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ENVIRONMENTAL POLLUTION BY LEAD AND OTHER METALS

Illinois University

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ENVIRONMENTAL POLLUTION BY LEAD AND OTHER METALS

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Progress Report

May 1, 1971, to April 30, 1972

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OF ENVIRONMENTAL POLLUTION
BY LEAD AND OTHER METALS

Sponsored by

NATIONAL SCIENCE FOUNDATION

IRRPOS GI-26
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ENVIRONMENTAL STUDIES PROGRAM

Graduate College

University of Illinois

at Urbana-Champaign

Progress Report

May 1, 1971, to April 30, 1972

CONTENTS

Page

Contents iii

Figures v

Tables xiii

Summary xvii

I. INTRODUCTION 1

Foreword 1

History and Organization 1

Objectives 5

II. RESEARCH PLAN 7

III. ANALYTICAL LABORATORY 17

A. Capabilities Expansion 19
B. Methods Development 20
C. Routine Processing Activities 23
D. Appendix 91
E. Personnel 107

IV. TOTAL ECOSYSTEM 109

A. Input Measures 110
B. Output Measures 114
C. Soil-Plant Sampling 122
D. Small Mammal 123
E. Invertebrate Work 128
F. Aquatic Communities 128
G. Personnel 136

V. AIR-SOIL-WATER-PLANT CONTINUUM 137

A. Aerosol Lead Particles 138
B. Movement of Lead in the Soil-Water System 164
C. Movement of Lead (Cadmium) in the Soil-Water-Organismal System 193
D. Personnel 234

VI. PLANT PROCESSES 239

A. Effects of Lead and Cadmium on Whole Plant Photosynthesis, Respiration and Transpiration of Dominant Species in the Watershed Ecosystem 240
B. Effect of Lead Salts on Photosynthetic Reactions in Isolated Chloroplasts 248

CONTENTS (cont'd)

Page

C. Respiratory Effects of Trace Elements. 259
D. Effects of Cadmium on Isolated Mitochondria. 267
E. Effects of Nickel on Isolated Mitochondria 283
F. Personnel. 290

VI. ANIMAL PROCESSES 293

A. Membrane-Lead Interactions 295
B. Studies Related to Heme Synthesis. 309
C. Accumulation and Release of Lead from Animal Tissue. 326
D. Personnel. 328

VIII. MODELING 331

A. Transport of Lead in the Environment 332
B. Dynamic Model of Lead Flow in the Body 360
C. Plant Modeling 366
D. Atmospheric Transport of Lead-Containing Particles 379
E. A General Systems Approach to the Economics of Environmental Quality Management: An Analysis of the Removal of Lead Additives from Gasoline. 384
F. Personnel. 404

IX. APPENDIX 407

A. Personnel. 407
B. Special Project Seminars. 411
C. Trace Metals Analysis Symposium, January 7 and 8, 1971 . . 412
D. Second Trace Metals Symposium, November 18 and 19, 1971. . 414
E. Seminar Series -- Chemistry and Biology of Trace Metals in the Environment, Spring Semester 1971 416
F. Lead Project -- Program Review Symposium 418
G. Project-Related Lectures and Publications. 420

FIGURES

Figure		Page
1	Multi-Interdisciplinary Organization	3
2	Present Structure	4
3	General Research Scheme	8
4	Typical Polarograms	23
4a	Typical Residual Current Curves	24
5	Typical Anodic Stripping Voltammograms for Various Sample Types	26
6	Typical Curves in Corn Grain	27
7	Typical Curves in Electrolyte with Mercury-Plated Rotating Wire Electrode	45
8	Typical Curves with Hanging Mercury Drop Electrode	46
9	Linear Potential Sweep Stripping of Lead Dioxide Deposited at Constant Overvoltage	61
10	Secondary Current Peak Charge Density vs. Deposition Time at Constant Overvoltage	62
11	Logarithmic Plot for First-Order Nucleation Kinetics . . .	63
12	Relationship Between Reversible and Secondary Peak Charge Densities	66
13	Cylindrical Tin Oxide Electrode and Cell Assembly	67
14	Lead Dioxide Deposition Rate as Function of pH	69
15	Lead Dioxide Deposition Rate as Function of Electrolysis Potential	70
16	Effect of Phosphate on Lead Dioxide Deposition and Stripping	73
17	Effect of Phosphate at Higher Lead Concentrations	74
18	Operational Flow Chart of Analytical Laboratory	84
19	Analytical Request/Report on Dual-Purpose Form	86
20	Analytical Request/Report on Dual Purpose Form	87
21	Schematic Diagram of Idealized Saline Branch Watershed . .	116

FIGURES
(cont'd)

Figure Page

22 Block Diagram of Water Sampler 117

23 Soil Lead Concentration in the Rural Subwatershed 124

24 Apparatus for Collection of Lead Halide Aerosol Particles from Controlled Temperature Melts 140

25 Apparatus for Reaction of Lead Halide Aerosols with Other Aerosols 144

26 Lead Bromide Particles Collected on Carbon Film 1 cm from Surface of Melt 146

27 Lead Bromide Particles Collected on Carbon Film 7 cm from Surface of Melt 147

28 Carbon Replicas of $PbBr_2$ Particles 148

29 Higher Magnification of $PbBr_2$ Carbon Replicas 149

30 PbBrCl Particles Collected on Quartz using Experimental Scheme of Figure 24 150

31 PbBrCl Similar to that in Figure 30 after Photodecomposition in Dry, CO_2 Free Nitrogen 151

32 PbBrCl Similar to that in Figure 30 after Photodecomposition in Dry, Free Air 152

33 PbBrCl Particles Caught on Carbon Film at Entrance of Reaction Chamber in Figure 25. 153

34 PbBrCl Collected on Quartz Slide Following the Experimental Scheme of Figure 25 155

35 PbBrCl Collected on Quartz Slide Following the Experimental Scheme of Figure 25 156

36 PbBrCl Collected on Quartz Slide showing Surface Morphology . . 157

37 PbBrCl Collected on Quartz Slide showing Surface Morphology . . 158

38 PbBrCl Collected on Quartz Slide showing Surface Morphology . . 159

39 Reeve-Angel 934AH Glass Fiber Filter 161

40 PbBrCl Collected on Glass Fiber Filter 162

41 Edgewise Cut of Glass Fiber Filter with PbBrCl Particles Collected on Upper Face 163

FIGURES
(cont'd)

Figure		Page
42	Photodecomposition of $PbBr_2$ in Air	166
43	Photodecomposition of PbBrCl	167
44	Lead Concentration with Depth in a Soil Column Following Leaching with Disodium EDTA	174
45	Lead Distribution in a Soil Column Following 2 Weeks of Continuous Leaching with Water	175
46	Lead Distribution in Soil Following Addition of Pb Acetate	177
47	Pb Removed from Corn and Sunflower Roots through a Series of Washes	194
48	Pulse Polarograms of 2 x 10^{-5}M Pb^{++} in 2 mM KNO_3	199
49	Effect of Freshly Detached Corn Roots with Time on Pb Concentration	200
50	Effect of Corn Root Exudate and EDTA on the Pulse Polarograms of Lead	203
51	Effect of Lead Concentration on the Pulse Polarograms of Lead in Corn Root Exudate Solution	206
52	The Uptake of Pb by Corn from Pb-Amended Hydroponic Solutions	211
53	The Uptake of Cd by Corn from Cd-Amended Hydroponic Solutions	213
54	Leaf Lead Concentrations Derived from a Soil Source	219
55	Stem Lead Concentrations Derived from a Soil Source	220
56	Root Lead Concentrations Derived from a Soil Source	222
57	Leaf Lead Concentrations Derived from a Nutrient Solution Source	223
58	Stem Lead Concentrations Derived from a Nutrient Solution Source	224
59	Root Lead Concentrations Derived from a Nutrient Solution Source	225
60	Lead Concentration in Earthworm Tissue	232
61	Net Photosynthesis, Dark Respiration, and Transpiration of Maize Plants	242
62	Effect of $CdCl_2$ on Photosynthesis and Transpiration of Sunflower Detached Single Leaves	244

FIGURES
(cont'd)

Figure		Page
63	Effect of $CdCl_2$ on Photosynthesis and Transpiration of Maize Detached Single Leaves	246
64	Effect of $CdCl_2$ at High Concentrations on Photosynthesis and Transpiration of Detached Single Leaves of Sunflower . .	247
65	Two Light Reactions in Photosynthesis	249
66	Rates of DCPIP Photoreduction in Isolated Chloroplasts as a Function of $PbCl_2$ Concentration	253
67	Relative Yield of Variable/Constant Fluorescence of Maize Chloroplast Suspensions as a Function of $PbCl_2$ Concentration.	255
68	Emission Spectra of Chl Fluorescence at 77°K of Normal and Treated Chloroplast Suspensions	257
69	The Effect of Varying $PbCl_2$ Concentrations on the Swelling of Isolated Mitochondria under Active Conditions	263
70	The Effect of $PbCl_2$ on Mitochondrial Swelling under Passive and Active Conditions	265
71	The Effect of Varying Concentrations of $CdCl_2$ on the Rate of Mitochondrial Respiration in the Absence of Phosphate . .	268
72	The Effect of Varying Concentrations of $CdCl_2$ on the Rate of Mitochondrial Respiration in the Presence of Phosphate . .	269
73	A Comparison of the Effects of $CdCl_2$ on the Rate of Electron Flow from Substrate to Oxygen and from Substrate to 2,6-dichlorophenolindophenol (DCPIP).	270
74	The Effect of Varying Time and Concentration Additions of Phosphate on the Rate of $CdCl_2$-Influenced Succinate Oxidation	272
75	The Effect of Phosphate on $CdCl_2$-Induced Changes in the Rate of NADH Oxidation and Concomitant Swelling	274
76	The Effect of Varying $CdCl_2$ Concentrations on the Swelling of Isolated Mitochondria under Active Conditions	276
77	The Effect of Varying Concentrations of Phosphate on the $CdCl_2$-Induced Swelling of Mitochondria Oxidizing Succinate .	277
78	The Effect of Varying Concentrations of Phosphate on the $CdCl_2$-Induced Swelling of Mitochondria Oxidizing Exogenous NADH	278
79	The Effect of Phosphate on the $CdCl_2$-Induced Increase in Mitochondrial Swelling under Passive Conditions	280

FIGURES
(cont'd)

Figure		Page
80	The Effect of Varying $CdCl_2$ Concentrations on Mitochondrial Coupling, expressed as Respiratory Control Ratios (RCR) . . .	281
81	The Effect of Varying $CdCl_2$ Concentrations on Mitochondrial Coupling, Expressed as ADP/O Ratios	282
82	The Effects of Phosphate on the $NiCl_2$-Induced Changes in Rates of O_2 Uptake	284
83	A Comparison of the Effects of $NiCl_2$ on the Rate of Electron Flow from Substrate to Oxygen and from Substrate to 2,6-dichlorophenolindophenol	285
84	The Effects of $NiCl_2$ on Swelling of Actively Respiring Mitochondria	286
85	The Effect of Varying $NiCl_2$ Concentrations on Mitochondrial Coupling Expressed as ADP/O Ratios	288
86	The Effect of Varying $NiCl_2$ Concentrations on Mitochondrial Coupling Expressed as Respiratory Control Ratios	289
87	Phospholipid Molecules	296
88	Membrane Conductance of Oxidized Cholesterol I and Oxidized Cholesterol II	297
89	Nernst Potential, RBC Bilayer Lipid Membrane	299
90	NMR Spectra of RBC Membrane Fragments in D_2O	300
91	NMR Spectra of RBC Lipid Extract in D_2O	301
92	Possible Conformations of Phospholipids in a Vesicle	305
93	Average Urinary ALA Excretion	315
94	Effect of Lead on Urinary ALA Excretion	317
95	Effect of Lead on Blood ALAD Activity	318
96	Effect of Lead on Liver ALAD Activity	319
97	Effect of Lead on Kidney ALAD Activity	320
98	Effect of Lead on Blood ALAS Activity	321
99	Effect of Lead on Liver ALAS Activity	322
100	Effect of Lead on Kidney ALAS Activity	323
101	Example of 1000 Sample Distribution Factors	334

FIGURES
(cont'd)

Figure		Page
102	Basic System Model	336
103	Basic Network Model	338
104	Basic Network Input Data	340
105	Basic Network Input Data (continued)	341
106	Seasonal Factors	342
107	Basic Network Output: Statistical Results	343
108	Basic Network Output: Plot of Atmosphere, Plants and Litter, Paved Surface, and Soil	344
109	Basic Network Output: Plot of Drainage Water, Aquatic Producers and Consumers	345
110	Basic Network Output: Plot of Water Outflow, Plant Export, Sediment and Soil Sinks	346
111	Expanded Network Model	350
112	Expanded Network Output: Statistical Results	351
113	Expanded Network Output: Plot of Herbivores, Carnivores, Soil Sinks (Zone I)	354
114	Expanded Network Output: Plot of Plants and Litter, Soil, Soil Water (Zone I)	355
115	Expanded Network Output: Plot of Plants and Litter, Soil, Soil Water (Zone II)	356
116	Expanded Network Output: Plot of Herbivores, Carnivores, Soil Sinks (Zone II)	357
117	Expanded Network Output: Plot of Atmosphere, Plants and Litter (Zone I)	358
118	Expanded Network Output: Plot of Soil Sinks (Zones I and II)	359
119	Physical Flow	362
120	Conceptual Model	362
121	Man-Model I	365
122	Man-Model II	365
123	Man-Model I	369

FIGURES
(cont'd)

Figures Page

124 Man-Model II 370

125 Variation with Time of K_{ij} 373

126 Seasonal Variations 373

127 Seasonal Variations 373

128 Plant Model 376

129 Lead Concentration (ppm) in the Root 380

130 Lead Concentration (ppm) in the Stem 381

131 Lead Concentration (ppm) in the Leaves 382

132 Systems Evaluation Framework 387

133 Systems Evaluation 'Checklist' 388

134 Gasoline-Lead Requirements, 1966-1980 400

MAP

1 Saline Branch Watershed 348

TABLES

Table — Page

1. Summary of Goals and Subgoals. 9
2. Research Program Activities. 12
3. Voltammetric Stripping Electrode Systems 32
4. Precision Estimates for Electrochemical Methods. 50
5. Definition of Detection Limits and Sensitivity for Selected Analytical Methods 52
6. Collection Efficiencies for Anodic Stripping Electrode 54
7. Comparison of Analytical Results by Atomic Absorption and Anodic-Stripping Voltammetry 78
8. Summary of Analytical Results of Round-Robin Interlaboratory Comparison 79
9. Analyses Performed between May 1, 1971, and May 1, 1972. . . . 89
10. Lead in Rainwater (ppb). 113
11. Lead in Rainwater (12-month period). 114
12. Lead Movement from Rural Section of Watershed (24-hour basis). 119
13. Lead Movement from Total Ecosystem Sewage Treatment Plant. . . 120
14. Lead Inputs and Outputs. 121
15. Lead Concentration in Small Mammals from Sites Within 10 m of a Heavy-Used Highway and Sites 200 m away from Roads. . . . 127
16. Aquatic Invertebrates in the Saline Branch of the Rural Subcompartment Taken in April 1972 131
17. Aquatic Invertebrates in the Saline Branch of the Rural Compartment Taken in April 1972. 132
18. Fish Taken from a Tributary to Saline Ditch in the Rural Subcompartment in April 1972 133
19. Fish Taken from the Saline Ditch (Urban Gage Site) in the Rural Compartment, April 1972 134
20. Mean Value of Lead and Number of Analyses of Aquatic Organisms from the Saline Ditch, March 1972. 135

Preceding page blank

TABLES (cont'd)

Table — Page

21. Decomposition of Lead Halides Subjected to Various Conditions. 165

22. Lead Concentrations with Depth in Soil Samples on the East Side of Route 45 near Thomasboro, Illinois 169

23. Characteristics of Surface Soils. 178

24. Mean Values of Pb Concentration in Drummer Silty Clay Loam as Determined with Several Procedures (ppm oven-dry soil weight) 180

25. Lead Concentration Means in Saybrook Silt Loam as Determined with Several Procedures 181

26. Means of 1 N HCl Extractable Pb with Depth in Saybrook Silt Loam Irrigated with Liquid Digested Sewage Sludge 184

27. Lead Concentration Means for Roadside Soil from Two Locations Near a Highway. 185

28. Equilibrium Studies of K^+ Saturated Montmorillonite with Pb^{++} in Solution 189

29. Equilibrium Studies of Ca^{++} Saturated Montmorillonite with PB^{++} in Solution. 190

30. Adsorption of Lead by Vermiculite 192

31. A Comparison of Pb Washed from Sunflower Roots to that Remaining in Roots After Washing. 196

32. A Comparison of Pb Washed from Corn Roots to That Remaining in Roots After Washing. 197

33. Soil Characteristics. 214

34. Lead and Cd Concentrations in Silt Loam Soils Used Experimentally. 231

35. Effect of $PbCl_2$ on the O_2 Uptake by Excised Corn Roots. . . . 260

36. The Uptake and/or Adsorption of Pb by Isolated Mitochondria . 266

37. The Uptake and/or Adsorption of Cd by Isolated Mitochondria . 273

38. Blood Incubation Study (Experiment 1) 312

TABLES (cont'd)

Table		Page
39.	Urinary ALA (Experiment 3).	314
40.	Heme Synthesis (Experiment 4)	314
41.	ALA Formed and Utilized in Blood and Excreted in Urine.	325
42.	Lead Storage in Animal Tissue	327
43.	Lead Input and Areas by Zones	349
44.	Concentrations (ppm) in Some Key Compartments by Zones at the End of Two Years	353
45.	Biological Parameters for Lead in Human Body.	367
46.	Man-Model I. Projected Accumulation	368
47.	Man-Model II. Projected Accumulation	369
48.	Automobile Emissions Standards.	392
49.	Environmental Protection Agency Phased Reduction Schedule . . .	397
50.	Tetraethyl Lead Content	402

SUMMARY

An interdisciplinary study of heavy metal pollution is currently in progress at the University of Illinois at Urbana-Champaign under a grant from the National Science Foundation RANN Program, a continuation of the IRRPOS Program. Objectives of the study are to determine the movements and effects of heavy metals (initially lead) in the environment. In order to facilitate the study, scientists from many disciplines are working together. Considerable progress has been made in all aspects of the study.

Analytical Laboratory

The activities of the analytical laboratory for the past year have centered around: laboratory remodeling and equipment acquisitions for expansion of capabilities, refining and development of routine analysis methods, and interlaboratory cooperations. In the period covered by this report, two remodeling phases have created 800 square feet of general purpose laboratory space and 600 square feet of space meeting minimal demands for emission spectroscopic analysis. Recent purchase of a second atomic absorption spectrophotometer of advanced type will double the output capacity for routine analysis as well as provide a useful tool for examining some of the factors influencing accuracy and precision of the method. Routine assay by atomic absorption has been improved by the addition of a sample metering pump to augment the more usual Bernouli aspiration of most AA burners. A beginning program in evaluating the effects of sample type on results has reduced the labor involved in second assays of the same type samples. Seven different anodic stripping electrode systems have been evaluated in a search for a replacement for the original equipment furnished with Environmental Science Associates Multiple Anodic Stripping Analysis unit. Two such

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electrodes have been shown to be superior to the ESA electrodes. A new cathodic stripping voltammetry method based on deposition of insoluble PbO_2 on the surface of a tin oxide (SnO_2) electrode has been investigated, proven to be useful and workable, and will shortly be used routinely for intermediate (10 to 100 ppb) analyses of samples inaccessible by other methods. Accuracy and precision estimates are being evaluated through two processes: interlaboratory "round robin" comparisons of representative samples and in-house cross comparison using two or more methods to assay the same routine sample. Results to date indicate the laboratory fares well in comparisons of round robin results with other cooperating RANN-supported project laboratories. In-house comparisons show no consistent bias between atomic absorption and anodic stripping based on some twenty results.

Total Ecosystem

Preliminary data on lead inputs, outputs, and other system fluxes have been obtained. Initial inputs to the 76-square-mile watershed-ecosystem are being determined by traffic volume counting and rainfall monitoring. Outputs from the system are primarily lead carried in stream water which is constantly sampled at four locations and agricultural harvest which has been intensively sampled. Initial input-output ratios are on the order of 100:1 which indicate a tremendous storage of lead within the system.

Considerable background information on lead concentrations and biomass of plants, animals, and the aquatic community has also been collected. These data indicate that in relation to total lead input to the ecosystem, only a small portion is contained in these components. Soil sampling has been completed for much of the ecosystem and appears to be the major storage component within the system. Sampling of all components will continue throughout the growing season.

Air-Soil-Water-Plant Continuum

Lead halide aerosols ($PbBr_2$ and $PbBrCl$) were generated from controlled temperature melts, characterized by microscopy, and found to be significantly more stable than lead halides in automobile exhaust.

Studies of soils revealed, as expected, significant accumulations in roadside areas. This lead was found to be generally immobile in soil columns, but exchangeable by Ca^{++} from clay surfaces. Similarly, lead was reversibly bound to exchange sites on corn roots, often to concentrations 5 to 10 times those within the root. Organic compound exudates from corn roots complexed lead and could be significant in mobilizing or immobilizing lead near the surface of the root.

Both lead and cadmium were taken up from hydroponic solutions by corn plants. At comparable solution concentrations cadmium was more toxic than lead, but concentrations in tissues were similar. Lead uptake studies from soils by tree seedlings indicated appreciable uptake, and, as was the case with corn, phosphate-deficient conditions prompted additional lead uptake. Considerable lead and cadmium were found associated with earthworms from lead and sludge-amended soils.

Plant Processes

Lead at concentrations up to 100 ppm significantly depresses net photosynthesis in maize plants. At 100 ppm lead, the rate of photosynthesis is only 45 percent of control. A slight increase in photosynthesis occurred at 200 ppm and another decline at 300 ppm. Transpiration was drastically reduced to zero at 100 ppm. Dark respiration followed a similar trend as net photosynthesis.

Cadmium supplied as $CdCl_2$ to sunflower and maize detached single leaves lowered photosynthesis and transpiration to essentially zero in some cases only a few minutes after it was introduced in the leaves.

Lead chloride seems to affect photosynthesis at the chloroplast level in several ways. From measurements of DCPIP photoreduction in chloroplasts and the time course of chlorophyll a fluorescence, lead chloride (50 μM to 500 μM) seems to inhibit photosystem II activity. However, at higher concentrations it probably feeds electrons to photosystem II. Measurements of emission spectra at 77°K of treated chloroplasts indicate that lead causes conformational changes of the membranes of the chloroplasts in such a way that spillover of excess light energy from PS II to PS I is decreased. Furthermore, lead chloride seems to affect the trap of photosystem II.

Elementary experiments on photosystem I activity suggest that some component of the crude preparation of the enzyme photosynthetic phosphopyridine neucleotide reductase seems to complex with lead chloride.

Animal Processes

Work of the Animal Processes Group has involved studies of lead interactions with a target organ tissue, the red blood cell (RBC) membrane, of lead influences on the metabolic pathway leading to heme synthesis and of lead accumulations in, and disappearance from, mammalian tissues.

The membrane studies employed whole isolated red cell membranes, lipid fractions of membranes, and pure phospholipids. Effects of lead on permeability, electrical conductivity, and nuclear magnetic resonance spectrum were investigated.

Bilayer membranes from RBC lipids do not change electrical conductivity in presence of 10^{-2} M $PbCl_2$ or KCl; they do, however, generate a Nernst potential at a tenfold $PbCl_2$ gradient, indicating that lead crosses the membrane. NMR spectra of the lipid extract membranes are not changed by lead but spectra of whole membranes are. Bilayer vesicles of phosphatidyl serine but not of phosphatidyl choline exhibited changed NMR spectra in presence of many cations. Lead fell into the series as expected in view of ion charge and size. If lead behaves in unique functions with membranes, it is probably in association with the protein portions of the membrane.

A series of studies relative to effects of lead on heme synthesis has shown: 1) that a lead-induced reduction in *in vitro* synthesis may be partially overcome by a mixture of vitamins C and B_{12} and by niacin when these vitamins are given at levels exceeding a normal intake to lead toxic rats, 2) that urinary excretion of amino levulinic acid (ALA) increases within hours after rats ingest lead, and 3) that this rapid initial increase of ALA excretion is a result of both stimulated synthesis and depressed utilization of blood ALA.

Preliminary data on tissue accumulation of lead in response to feeding diets containing 20 to 320 ppm lead to adult rats indicate that skeleton and kidney accumulate the highest concentrations of lead, amounting to thirty- and sevenfold increases, respectively, after four weeks of feeding.

Modeling

Initial efforts of the modeling team have concentrated in four basic areas including total ecosystem transport modeling, plant and animal uptake, and distribution modeling and economic systems modeling. Preliminary models have been developed and are being refined as field or laboratory data become available. Air transport modeling is currently being developed.

I. INTRODUCTION

FOREWORD

This report constitutes a progress report of research conducted under the IRRPOS program of the National Science Foundation during the final portion of the exploratory and planning phase of the project from May 1, 1971, to October 31, 1971, and the first six months of the research phase of the project under the RANN program of the National Science Foundation from November 1, 1971, to April 30, 1972.

Experimental findings and conclusions should be regarded as tentative and should not be cited, reproduced, or included in other publications without the express approval of the project director.

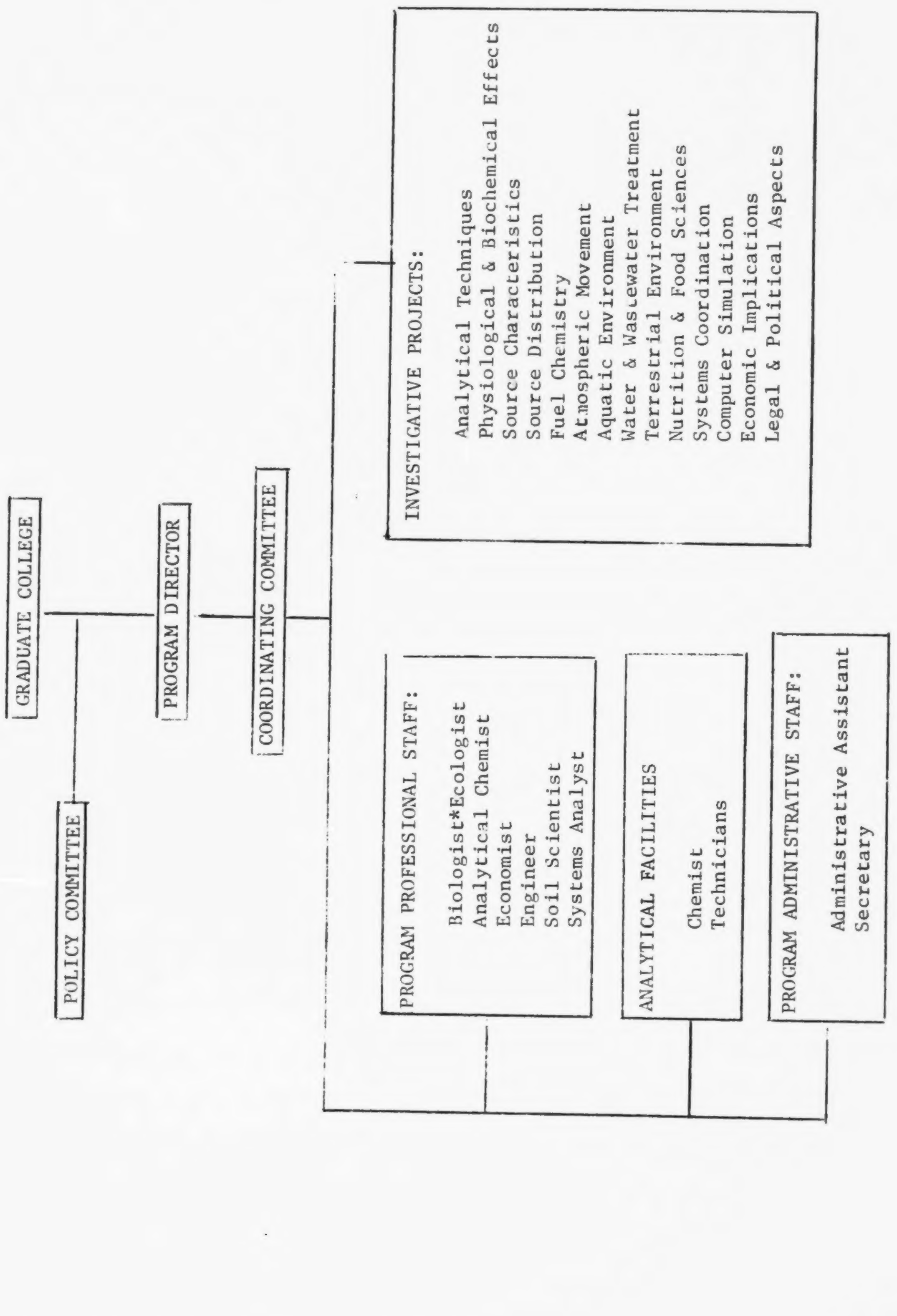
HISTORY AND ORGANIZATION

The concept of a wide-scale interdisciplinary project on the Environmental Effects of Pollution by Lead originated in 1968 with Professor R. L. Metcalf, Head of the Department of Zoology and Professor of Entomology. Professor Metcalf, together with Professor Ben B. Ewing, Director of the Water Resources Center and Professor of Civil Engineering, discussed the proposed project with faculty members from many departments in several colleges of the University. By the autumn of 1969, an interdisciplinary Coordinating Committee had been appointed to develop a research plan. This Coordinating Committee formed the nucleus of an ad hoc committee on environmental studies appointed by Vice Chancellor H. E. Carter in February 1970 to advise the University administration as to the organization of a campus-wide unit to be responsible for all phases of environmental studies. This committee in July 1970 recommended the formation of an Institute for

Environmental Studies to report directly to the Office of the Chancellor in parallel with the various colleges and other campus-wide institutes. The concept of an Institute of Environmental Studies was approved by the Faculty Senate of the Urbana-Champaign campus in December 1970, but a full year was to pass before the Senates of the other two campuses had acted and formulated a plan of integration of environmental studies among the three campuses. The Board of Trustees of the University of Illinois approved the establishment of the Institute, and it has now been submitted to the Illinois State Board of Higher Education for final approval. In the meantime, an Environmental Studies Program was set up under the Graduate College of the Champaign-Urbana campus, and Professor B. B. Ewing was appointed Director of the Program in March 1971.

The present research project gradually developed from twenty-two separate research proposals from various departments and centering around the Environmental Effects of Lead into a single interdisciplinary study of Environmental Pollution by Lead and Other Metals. Evolution of the project continued through the exploratory and planning phase which was supported by a grant from the IRRPOS program of the National Science Foundation from July 1, 1970, through October 31, 1971. Initially conceived as a mixed multidisciplinary-interdisciplinary project (Figure 1), it has gradually changed into a truly interdisciplinary organization. The present structure of the project is indicated in Figure 2. In this structure, the Coordinating Committee retains its function as an interdisciplinary policy-making group charged with the responsibility for any major changes in research plan or emphasis. The Steering Committee, on the other hand, is made up of the Group Leaders of the six research groups. Of these, three are laboratory-oriented groups as follows:

TENTATIVE ADMINISTRATIVE STRUCTURE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COORDINATING COMMITTEE
INVESTIGATIVE PROJECTS:
Analytical Techniques
Physiological & Biochemical Effects
Source Characteristics
Source Distribution
Fuel Chemistry
Atmospheric Movement
Aquatic Environment
Water & Wastewater Treatment
Terrestrial Environment
Nutrition & Food Sciences
Systems Coordination
Computer Simulation
Economic Implications
Legal & Political Aspects
PROGRAM PROFESSIONAL STAFF:
Biologist*Ecologist
Analytical Chemist
Economist
Engineer
Soil Scientist
Systems Analyst
ANALYTICAL FACILITIES
Chemist
Technicians
PROGRAM ADMINISTRATIVE STAFF:
Administrative Assistant
Secretary

Figure 1. Multi-Interdisciplinary Organization

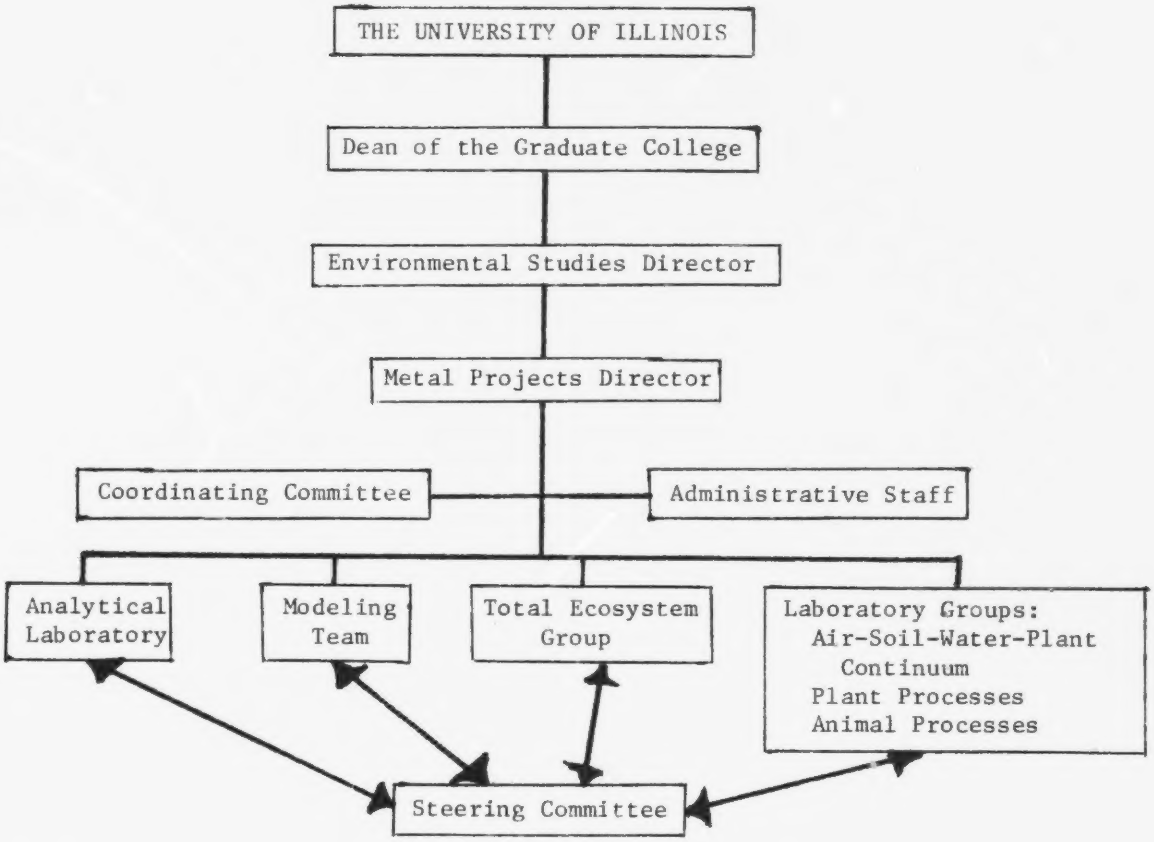
THE UNIVERSITY OF ILLINOIS
Dean of the Graduate College
Environmental Studies Director
Metal Projects Director
Coordinating Committee
Administrative Staff
Analytical Laboratory
Modeling Team
Total Ecosystem Group
Laboratory Groups: Air-Soil-Water-Plant Continuum Plant Processes Animal Processes
Steering Committee

Figure 2. Present Structure

Air-Soil-Water-Plant Continuum Group (H. A. Laitinen, Group Leader)
Plant Processes Group (F. A. Bazzaz, Group Leader)
Animal Processes Group (R. M. Forbes, Group Leader).

Total Ecosystem Group (G. L. Rolfe, Group Leader)
Modeling Team (G. L. Rolfe, Group Leader) and
Analytical Laboratory (A. M. Hartley, Group Leader)

comprise the other three groups. While each group is itself interdisciplinary in character, it is the function of the Steering Committee to foster communication among groups and thereby weld the project more effectively into a single interdisciplinary team.

Although the project is oriented toward research, there is an important educational component as well. Three students have thus far received their Ph.D. degrees presenting theses with a substantial component of research relevant to the project. Twenty-eight students have been employed thus far as research assistants. In addition, postdoctoral associates (who can be regarded as interns with regard to environmental science) and B.S. and M.S. level research assistants (who are not students formally) are receiving valuable job experience in gathering, recording, and preparing samples and performing analyses.

OBJECTIVES

The Heavy Metals Task Force of the Environmental Studies Program constitutes a research effort of wide interdisciplinary scope to marshall the intellectual resources of the University of Illinois to attack an environmental problem, and to provide for the assessment of alternatives for the solution of various aspects of the problem. Although the primary thrust of the research has been toward the environmental effects of lead, the interdisciplinary team, the scientific approach, and the methodology adapt themselves to a more general study of the environmental effects of toxic trace elements.

To gain an understanding of the behavior of heavy metals in the environment, the initial attack has been to assess the occurrence of lead in an ecosystem consisting of a watershed drainage system, encompassing both rural and urban components. Mathematical models are being developed to describe the transport of trace metal pollution through various components of the environment at various organizational levels ranging from the total ecosystem, its major components, organisms, organs, cells, and membranes through to the molecular level. Lead was chosen as the prime pollutant for this study because it is so widespread throughout the environment that it can be purposefully studied at all organizational levels. Various components of the study will lend themselves to other metals of lesser distribution.

An important ultimate objective of the study is to evaluate alternatives for the control of a pollutant by a variety of objective criteria, and to assess alternatives so that decision making for environmental improvement may be more rational.

An additional motivation for undertaking this research has been to develop an effective organization and management of an interdisciplinary study of societal impact in an academic setting to achieve both short-range goals and long-range contributions to human knowledge.

RESEARCH PLAN

The ultimate objective of the research program is to develop an understanding of the movement and effect of lead and other trace metals in the environment so that decisions regarding control of these possible pollutants might be made more rationally. A general research plan for accomplishing this ultimate objective was developed and is present in Figure 3. The symbol "X" represents accomplishments during the exploratory and planning phase, while the symbol "O" represents planned research. A third symbol, "Z", has been added to the diagram representing research initiated or completed during the first six months of the new grant. Considerable progress has been made toward moving along the research plan to the ultimate objective of rational long-range planning.

In order to understand the movements of metals in the environment we have defined a terrestrial ecosystem typical of many areas of the midwest as a focal point. To understand the transport of metals in the ecosystem and their effects on system components, we have identified five primary goals, each with a number of principal subgoals. These are listed in Table 1.

The first goal is the understanding and modeling of mass transport of metals in the terrestrial ecosystem. Models for understanding these movements are being developed and are continuously refined as new data become available. The initial mass transport model being developed is for a terrestrial ecosystem based on the Saline Branch Watershed in Champaign County, Illinois. A complete description of the model can be found under the Modeling section of this report. The system is presently defined and very well mapped.

	ASPECTS OF MODELING	PHASE I System Definition	PHASE II System Mapping	PHASE III System Behavior	PHASE IV System Model	PHASE V System Simulation
MAJOR SYSTEMS	A. Industrial Distribution System	X 0000	0000	000	0	
	B. Terrestrial Ecosystem	XXX Z XXXX 0	XXXX Z XXXXX 00	ZZ 0 XXX 0000	Z 00	0
	C. Aquatic Ecosystem	ZZ XXX 000	Z XXX 000	000	0	
	D. Human System	0000	0000	X 0		
SUBSYSTEM PARAMETERS	X Mass Transport	X Z XXXX	XX Z XXXX	ZZ 0000	Z 0000	0
	Y System Response					
	1. Physical & Chemical Effects on Environmental Elements	Z XXX 0	XXXX Z XXXX 0	Z X 00	0	
	2. Biochemical & Physiological Effects on Biological Elements					
	(p) Plants	XX Z XXXX 0	XX ZZ XXXX 00	Z 0 XX 0000	0000	0
	(a) Animals	XX Z XXXX 0	X Z XXXX 00	X 0000	000	
	(h) Humans	00	00	X 00	0	
	Z Societal Impacts					
	1. Human Welfare	Z		X 00		
	2. Economics	Z		00	0	0
	3. Social Values	Z				
	SOCIETAL DECISIONS	POLLUTION CONTROL	DEGRADATION PREVENTION	TECHNOLOGY ASSESSMENT	LONG-RANGE PLANNING	

Figure 3. General Research Scheme

TABLE 1

Summary of Goals and Subgoals

I. Mass transport in the Terrestrial Ecosystem
  A. Develop Input-Output Models of Total Ecosystem
  B. Air-Soil-Water-Plant Continuum Study
  C. Animal Accumulation Studies
  D. Aquatic Compartments of Watershed
  E. Synthesis of Submodels into Integrated Models

II. Effects on Plants and Animals (Terrestrial and Aquatic)
  A. Plant Effects
  B. Animal Effects
  C. Integration with Mass Transport Models

III. Production and Consumption of Products Containing Lead and Other Metals
  A. Production and Distribution of Lead Products
  B. Consumption of Lead Products
  C. Disposal of Waste Residuals Generated from Consumption of Lead Products
  D. Production, Distribution, and Consumption of Products that Contain Similar Metals of Interest

IV. Net Effect on Social Welfare from Environmental Pollution by Lead
  A. Effects on Social Welfare from Consumption of Lead Products
  B. Effects on Social Welfare from Consumption of Goods Contaminated by Lead
  C. Effects on Social Welfare from Consumption by Substitute Products
  D. Effects on Social Welfare from Production of Contaminated Products

V. Conceptual Framework for Use of Models for Decision-making by Governmental Agencies

Most present research is concerned with understanding system behavior and further development of the system model. We are currently developing a more realistic input-output model based on traffic data which provide a better estimate of lead input to the system and stream data which provide a better estimate of lead output from the system. Present estimates of the magnitude of the inputs and outputs of the system can be seen in Table 14 of the Total Ecosystem section. Accumulations or depletions within the ecosystem are currently being inventoried by sampling of the principal components, i.e., soil, plants, and animals, etc.

The Air-Soil-Water-Plant Continuum, animal accumulation studies, and aquatic compartment studies are being intensively studied by interdisciplinary working groups.

The second primary goal is to study the biological effects of heavy metals on life processes of plants and animals. Investigations of these effects are proceeding at several organizational levels including organism, organ, cell, and sub-cellular.

Two additional primary goals relate to the better understanding of the industrial production-consumption system for goods containing lead and other metals. One is to assess and model the determinants of social welfare within an economic system which controls the interindustry production, distribution, consumption, and disposal of goods containing heavy metals. The other is to assess the net effect on social welfare associated with environmental pollution. Progress has been made toward evaluation of both of these major goals.

The fifth major goal is to develop conceptual frameworks for the use of models for assisting management and planning for decision-making by public and private agencies.

Table 2 is a list of research program activities necessary for attaining each of the goals and subgoals presently viewed by the research team. Those activities currently initiated ("I") or completed ("C") are designated in the table. Research program activities are continuously revised to attain project goals.

TABLE 2

Research Program Activities

I. Mass Transport in Terrestrial Ecosystem

A. Mass Transport Studies of Total Ecosystem

1. Inputs

I a. Study air sampling methods and design network (p. 95)*
I,C b. Plan meteorological monitoring network (p. 95)
I,C c. Plan traffic monitoring network (p. 95)
d. Set up air sampling network (p. 96)
I e. Set up meteorological monitoring network (p. 96)
I f. Set up traffic monitoring network (p. 95)
I g. Data collection--air sampling (p. 95)
I h. Data collection--meteorological (p. 96)
I i. Data collection--traffic (p. 95)
I j. Begin collection of influent, effluent, and sludge from sewage treatment plant (p. 101)
I k. Begin study of lead deposition (fallout and rainout) in watershed--correlate results with map of residual levels over the watershed (pp. 96-97)
l. Begin study of other possible Pb inputs such as industrial and residential discharges
m. Begin investigation of possible inputs of heavy metals other than Pb to the system (p. 89)
I n. Begin subdividing watershed into smaller compartments based on land use (intensive monitoring of these compartments) (pp. 89-90, 102)

2. Outputs

I,C a. Construct and test prototype water sampler (p. 102)
I b. Develop water sampling methodology (p. 102)
I c. Begin water-level recording at stream-gaging locations (p. 102)
I,C d. Construct stream-gaging station and activate others (p. 102)
I e. Begin calibration of gages (p. 102)
I f. Begin collection of water samples at all stream gages (p. 102)
g. Construct flume for rural subwatershed--set up portable sampler and begin collecting water samples (p. 102)
h. Begin study of discharges from sanitary landfill (p. 101)
I i. Determine percentages of Pb moving out of system as sediment load at various stream flow rates--also "drift" of plankton out of system (p. 103)
I j. Study output of Pb through crop harvest (pp. 98-99)
I k. Determine possible output through animal exports (pp. 100, 103)
l. Begin study of outputs of other heavy metals (p. 89)

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* Page numbers refer to Proposal to National Science Foundation dated June 1971

3. Synthesis

I a. Develop preliminary Input-Output Model for watershed--based on stream-gaging, traffic monitoring, air sampling, and meteorological data collected to date (pp. 69-72)
b. Evaluate and refine preliminary Input-Output Model (pp. 69-72)
c. Develop Input-Output Models for subwatersheds and compare (pp. 69-72)
d. Continue refinement of preliminary models (pp. 69-72)

B. Soil-Water-Plant Studies

I 1. Complete collection and analysis of background soil, water, and plant samples from watershed compartments (mapping) (pp. 105-108)
2. From (1.), plot a spatial distribution map of Pb in the watershed
I 3. Define system for other heavy metals (p. 89)
4. Begin mapping of system for other heavy metals (p. 89)
C 5. Complete studies of lead uptake by tree species (pp. 99, 107, 116)
C 6. Determine gross location of Pb in the plant (tree species) (p. 116)
I 7. Initiate studies of coordination capacity of soil-water system to combine with heavy metals and rate of release of reactive species (p. 109)
I 8. Initiate studies to determine organic and inorganic forms of Pb present in soil, sediment, and water (p. 114)
I 9. Initiate studies of relationship between coordination capacity, form of Pb, and plant uptake (p. 116) I
I 10. Initiate study to determine the effects of complementary ions, such as phosphate, carbonate, and sulfate on Pb uptake (p. 116)
11. Develop preliminary mass transport model for soil-water-plant system (pp. 69-72)

C. Animal Accumulation Studies

I 1. Initiate studies to determine the ultimate distribution of inhaled and ingested Pb in body tissues of domestic animals or suitable laboratory test animals (p. 119)
I 2. Begin planning study of wild animal components (pp. 121-127)
I 3. Begin collecting representative samples of wild animals (small mammals, birds, and insects) from the various compartments of the watershed for analysis (pp. 121-122)
4. Wild animals found to be accumulating significant quantities of Pb will be selected for body distribution studies as described in (1.)
I 5. Determine natural dose level (ingested, inhaled, and absorbed) for those animals described in (1.) and (4.) (pp. 119, 122-127)
I 6. Develop preliminary animal accumulation model (pp. 69-72)

D. Aquatic Compartments of Watershed

I 1. Establish food chains and component organisms (Saline Branch Stream)
I 2. Establish Pb levels in organisms (Saline Branch Stream)
3. Determine rate of uptake and possible concentration factors--related to work on activity of metals in natural waters (I, B, activities 7, 8, 9, and 10) (pp. 103, 104, 109-118, 141, 142)
4. Develop preliminary model of Saline Branch Stream (mass transport) (pp. 69-72)
5. Evaluate and refine preliminary model (pp. 69-72)

E. Synthesis of Submodels into Integrated Models

II. Effects of Metals on Plants and Animals (Aquatic Terrestrial)

A. Plant Response Studies

1. I Initiate studies of Pb effects on Ps, Rs, and Ts of the whole plant (pp. 105-108)
2. I Begin development of monodispersed aerosol generator for plant and animal studies
3. I Initiate studies to determine at the plant cellular and subcellular level where Pb and selected other heavy metals accumulate (pp. 116-117)
4. I Initiate studies to determine and evaluate the effects of Pb on the mechanism of Ps, on mitochondrial reactions, and their sites of reactivity (pp. 117-118)
5. Begin to develop preliminary models relating lead level to plant mechanism responses (pp. 69-72)

B. Animal Response Studies

1. I Using information from activities 1, 3, 4, and 5 of I, C and literature, determine organs most likely to be affected by Pb
2. I After critical organs are determined and test species are selected, begin study of mechanisms of Pb effect on these organs (cellular and subcellular levels) (pp. 129-131)
3. Develop preliminary models relating lead retention levels in critical organs to effects (pp. 69-72)
4. Evaluate and refine preliminary models (pp. 69-72)

C. Integration with Mass Transport Models

III. The Interindustry System for Production and Consumption of Lead Products

A. Production and Distribution of Lead Products

1. Define and map the industrial production and distribution system

    - I a. Define and map a national interindustry mass transport system for the lead industry and for the major industrial users of lead
    - I b. From the national system, define regional interindustry systems from those regions that are extremely dependent on the lead industry as a source of income and employment
    - I c. Map the interindustry mass transport systems into a model that will predict the changes in industry employment and regional income that result from changes in the final demand for products which contain lead as an essential ingredient

2. Study the behavior of the lead industry

    - I a. Define the interrelationships between the market behavior of the firms in the lead industry and the structural determinant of industry supply
    - I b. On the basis of these interrelationships, predict changes in the structure of the industry that result from changes in the final demand for lead products

B. Consumption of Lead Products

I 1. Define the economic subsystem for the consumption of products that contain lead as a technically expedient ingredient
2. Map the regional economic subsystem for the consumption of these products
3. Study the behavior of the regional economic subsystem and build models for that behavior
I 4. Simulate the system under various alternatives

C. Disposal of Waste Residuals Generated from Consumption of Lead Products

I 1. Define the interface between the economic subsystem for consumption and the environmental subsystem within the economic-environmental mass transport system for lead
I 2. Map the economic and environmental subsystems along the interface
3. Determine the behavioral characteristics of the economic and environmental subsystems along this interface
4. Simulate the interaction between the economic and environmental subsystems along the interface for prediction of the flow of wastes to the environment under alternative economic controls

D. Study Production, Distribution, and Consumption of Products that Contain Similar Metals of Interest

IV. The Net Effect on Social Welfare Associated with Environmental Pollution from Lead. The Net Effect is Determined by the Difference Between Positive Effects from Consuming Lead Products and Negative Effects arising from Pollution.

A. Evaluate Effects on Social Welfare Resulting from Consumption of Lead Products

I 1. Determine the performance characteristics for the consumption of goods and services associated with goods that contain lead as a technically expedient ingredient
2. Map the performance characteristics into utility spaces for several representative consumers within the economic subsystem
I 3. Determine the consequences to human health resulting from the consumption of goods and services associated with goods that contain lead as a technically expedient ingredient
4. Map these health effects into utility spaces for several representative consumers within the economic subsystem

B. Evaluate Effects on Social Welfare Resulting from Consumption of Goods Contaminated by Lead

1. Determine the performance characteristics for the consumption of goods and services associated with goods that contain lead as an unintentional ingredient as a result of environmental contamination of the goods or the raw materials from which they are produced
2. Map these performance characteristics into utility spaces for several representative consumers

  3. Determine the consequences to human health resulting from the consumption of contaminated products and goods
  4. Map these health effects into utility spaces

- C. Evaluate Effects on Social Welfare Resulting from Consumption of Substitute Products

I

  1. Determine the performance characteristics for consumption of goods and services associated with goods that do not contain lead but that are considered as replacements for those goods and services that do contain lead
  2. Map these performance characteristics into utility spaces
  3. Determine the consequences to human health associated with consumption of these substitute products
  4. Map these health effects into utility spaces

- D. Evaluate Effects on Social Welfare Resulting from Production of Contaminated Products

  1. Using the simulation models generated in III C 4 above, assess the economic impact of the environmental response to the lead that is introduced into the environment as a waste emission from economic activity
  2. Using the simulation models, assess the economic impact of restricting the quantity of lead distribution and accumulation in the environment stemming from waste emissions
  3. Using the simulation models, assess the economic impact of removing the lead that is already moving through the ecosystem

I V. Develop a Conceptual Framework for the Use of Models for Assisting Management and Planning for Decision-making by Governmental Agencies

III. ANALYTICAL LABORATORY

INTRODUCTION

During the period 1 May 1971 to 1 May 1972, the major activities of the Analytical Laboratory have been the completion of analyses relating to work supported by planning grant GI 26, solidification of methodology and advance planning for work to be done under grant GI 31605, and, beginning approximately in March 1972, analytical support for the present research efforts. Ancillary activities such as methods development, equipment fabrication and testing, and extra-laboratory interactions ("round robin") analyses have been accomplished by working in and around the routine sample flow patterns.

Interaction of the Laboratory with its users has been and will continue to be evolutionary in form. During the initial planning grant period, interactions tended to be individualistic because the several projects requiring analytical services had little planned relation. Analyses for each project had to be self-consistent but not of such form as to permit ready intercomparisons with other projects.

With the beginning of the present project, with its high degree of cooperative research, it was readily apparent that results emanating from this laboratory had to be as free of sample effects and biases as possible because a stated object of the program was to compare results from all groups involved. A continuing objective of the laboratory has been to discover and, if possible, remove analytical biases resulting from the uniquenesses of sample types. Much work has gone into developing a catalogue of so-called "Matrix Factors" in atomic absorption spectroscopy as a first step in putting all reported results on an equal footing.

For the same reasons, considerable effort has been devoted to development of alternative voltammetric electrode substrates for stripping analysis to achieve a higher degree of reliability than has been customary using the graphite electrodes developed by Dr. Wayne Matson and sold by Environmental Science Associates. Delineation of at least two superior alternative anodic stripping electrodes have been accomplished, as well as a novel cathodic stripping electrode based on the semi-conductor, SnO_2. Recent samples arising from the Animal Processes group in the form of rat tissues (blood, muscle, kidney, liver, etc.) have afforded the opportunity of intercomparison of electrochemical and atomic absorption methods on the same samples. This work will continue to provide a useful source of in-house comparisons between methods in the course of routine sample analysis.

Interactions between the Laboratory staff and the several research groups has become more extensive for inter-relating the work of one group with that of another. The mechanism of frequent "group meetings" of the several groups (Animal Processes, Plant Processes, Total Ecosystem, and Modeling) provides the ready route to close cooperation between each group and the Laboratory by including Mr. Raymond Vogel, Assistant Director of the Laboratory, in all the group meetings. In this fashion, questions relating to analytical requirements and performance, sample size, sampling frequency, sample flow and results reporting are handled weekly in an *ad hoc* fashion. Overall interaction between the Laboratory and the research groups with regard to priorities and other questions is a function of a recently-created Steering Committee of which the Laboratory director, Dr. Hartley, is a member.

A. Capabilities Expansion

1. Atomic Absorption Spectrometry

Space has been prepared and the necessary services installed for a second atomic absorption spectrophotometer to provide for the additional analytical load scheduled during the spring and summer months. The spectrophotometer, a Jarrell-Ash Model 810, was delivered early in May. In addition to use in routine service analyses, the instrument provides capabilities for development of more efficient means to correct for matrix effect and background absorption, especially in the analysis of animal tissues.

2. Electrochemical Methods

In December 1971, a 200 ft^2 room on the second floor of the Environmental Research Laboratory was renovated and converted to an electroanalytical laboratory, thus separating this function from the general laboratory and spectrophotometric operations. This is desirable for electrical isolation and to minimize chemical contamination problems in determination of heavy metals in the parts-per-billion range.

Equipment, for the most part obtained by loan and transfer from the School of Chemical Sciences, was originally acquired by Dr. Hartley during his tenure on that School staff. The laboratory is equipped for routine electrochemical assays except for rapid events. Operational electroanalytical equipment includes a Sargent Model XV Recording Polarograph, a Fisher-Kelly type vacuum tube operational amplifier polarograph, two Philbrick solid-state multi-functional electrochemical units, three Heath operational amplifiers severally fitted with polarographic, cyclic voltammetric or chronopotentiometric control consoles, and an Environmental Science Associates MASA 2014 Multiple Anodic Stripping Assembly.

3. Emission Spectroscopy

Plans were drawn up and allocation of approximately $17,000 of University funds approved for remodeling approximately 800 ft^2 of space in the Environmental Research Laboratory to provide facilities for installation of an emission spectrographic laboratory suitable for the determination of trace heavy metals in environmental materials. The spectrograph, a Hilger Large Littrow prism type, and an arc/spark power supply unit were obtained by transfer from the Department of Agronomy and the College of Engineering. A microphotometer and equipment for photoprocessing have been purchased.

Construction of the necessary partitions, installation of plumbing and electrical facilities, ductwork, drop ceiling and floor tile have been essentially completed. Installation of the required air-conducting and heating systems remainsthe last principal item to complete the project.

Emission spectroscopy will provide needed matrix information for methods development in atomic absorption spectroscopy. Emission spectroscopy will also be employed for routine analyses when matrix effects make atomic absorption measurements impractical, or when multiple-element analysis is needed. High priority applications will be in the multi-element analysis of soil and for animal tissues such as bone and blood.

B. Methods Development

During the exploratory and planning phase of the Program, the Analytical Laboratory carried on method development to supply trace analyses for heavy metals on a routine basis of a wide variety of materials originating from plant, animal, soil, water and synthetic systems. It was realized that complexity of sample preparation would need to be kept to a minimum to minimize sample contamination and also to permit employment of part-time operating personnel of experience and background less than would usually be the case in a trace substances analytical laboratory.

Procedures developed according to these needs followed a relatively simple two-step preparation applicable to samples of plant and animal origin, soils, and other environmental materials. The sample preparation procedure involves only dry-ashing of a preweighed sample, followed by digestion in 3 N HCl. The analytical measurement is then made on the resultant solution by atomic absorption spectroscopy when the estimated lead concentration is above 0.3 ppm in the analytical solution, and by anodic stripping voltammetry at lead concentrations below that value.

1. Atomic Absorption Spectrometry

In the analytical determination of lead and other heavy metals by atomic absorption spectrometry, the presence of the sample matrix constituents in the nebulized analytical solution causes a suppression of the analytical signal as compared to like concentrations of lead in pure aqueous standards. During the first phase of the Program, preliminary results had been obtained on the magnitude of this so-called "matrix effect" for several sample types, and preliminary values for corresponding "matrix factors" were generated which, when multiplied by the observed lead values relative to pure water standards, would approximate the lead value obtained by "standard addition" to that sample.

Work since May 1971 has continued in the investigation of analytical variables of matrix effect, and the relative contributions of the principal variables were evaluated. Through a combination of analytical development/service operations, matrix-factor values for thirty-one different matrices of of animal, plant and soil sample types have been generated and put into use in service analysis operations. The use of these factors in combination with control of preliminary sample preparation procedures used by investigators and in-house with regard to sample and reagent concentrations in the analytical solution will permit more efficient use of simple aqueous standards in the analysis of a wide variety of sample types, with consequent reduction in the number of analytical measurements required per analysis.

a. Matrix-Effect Variables. The term "matrix effect" is a loosely-defined catch-all term which refers to the change in response of an analytical signal caused by the presence of sample-matrix constituents in the preparation on which the analytical measurement is made.

Matrix effect exists to some degree in every analytical method, but is especially significant in flame spectrometry. In the determination of lead in biological materials by atomic absorption spectrometry, two variables of matrix effect can readily be observed and evaluated: 1) background absorption, an additive factor, and 2) sensitivity reduction relative to aqueous standards, a proportionality factor. The combined effect of these factors is shown in Figure 4 reproduced from strip charts of atomic absorption data output in the analysis of lead in the prepared crayfish sample. The total absorption signal, $A_{Pb + Bkg}$, from the sample measured at a wavelength of 283.3 nm consists of the sum of absorbance, A_{Pb}, specific to lead, plus the non-specific (background) absorption, A_{Bkg}. The lead concentration in the sample, determined by standard addition, is 1.0 parts per million. The ratio between the absorbance of a 1-ppm aqueous lead standard and the background-corrected absorbance of the crayfish sample solution is the "matrix factor"

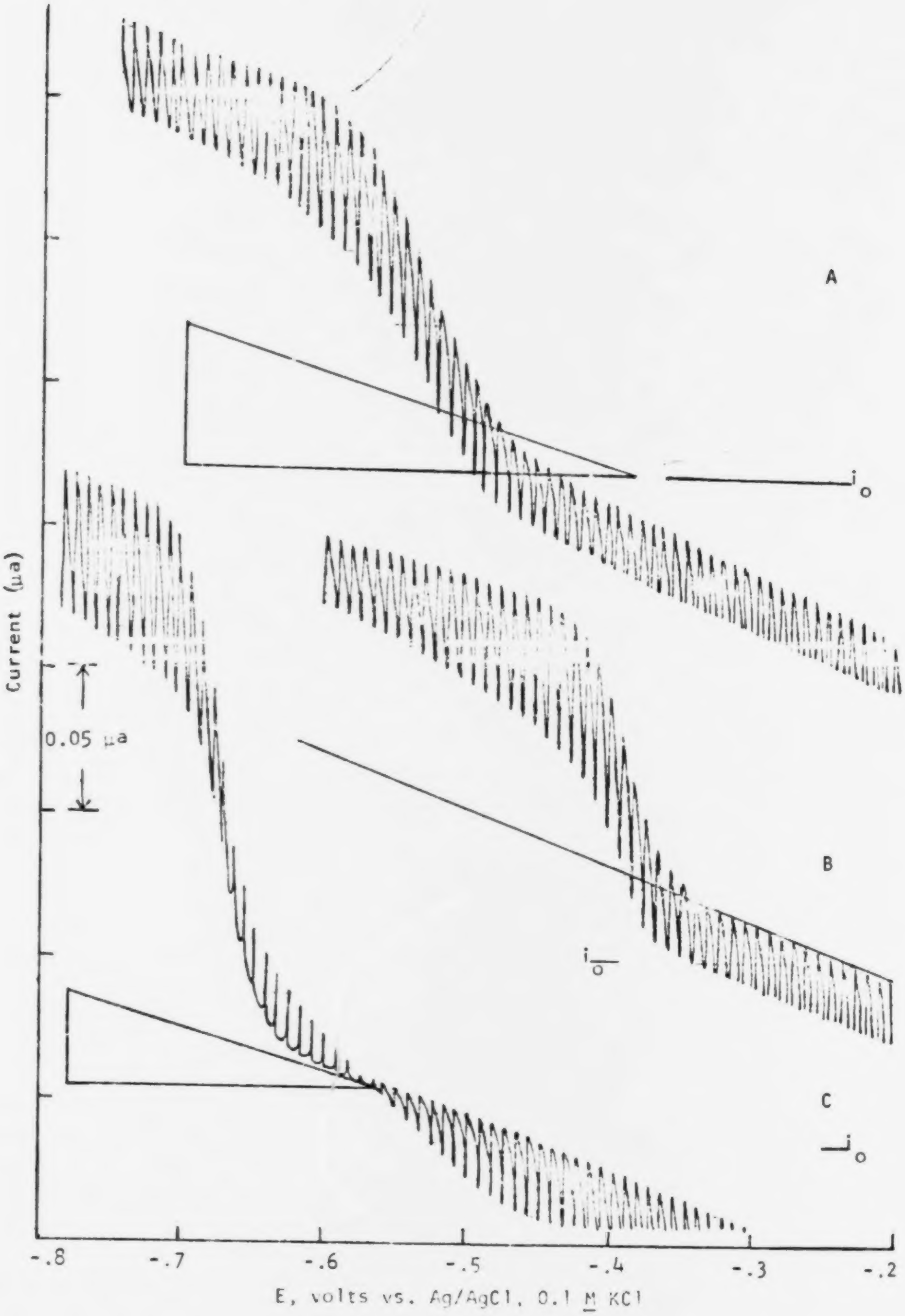


Figure 4. Typical polarograms for 2 ppm (A) Thallium; (B) Lead; (C) Cadmium in 0.1 M KCl electrolyte. Instrument output is RC damped with time constants: (A) and (C), 0.25 sec; (B) 0.65 sec.

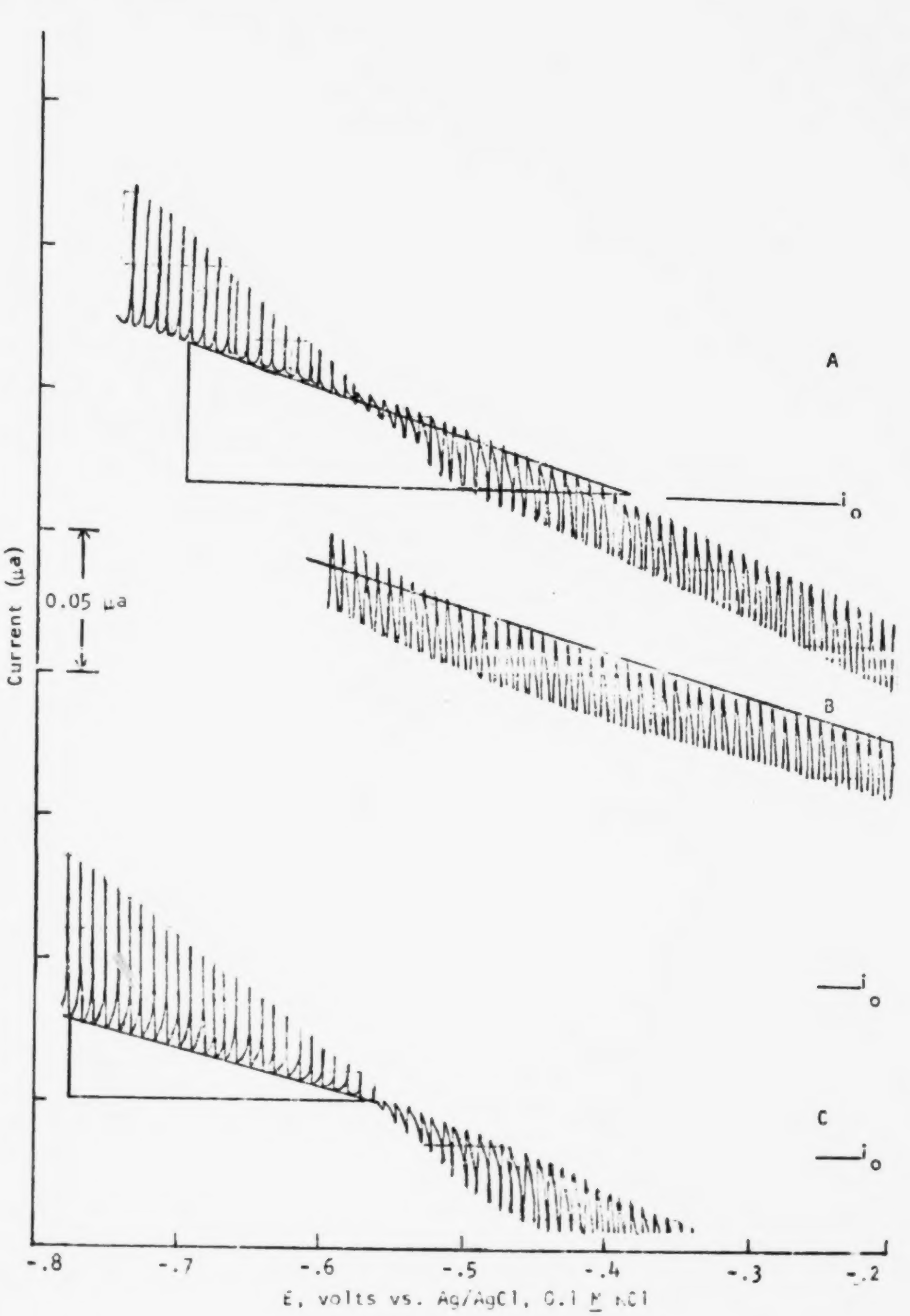


Figure 4a. Typical residual current curves for 0.1 $\underline{M}$ electrolyte. Damping is identical to the corresponding curve in Figure 4.

which in this case has a value of 1.4. In subsequent analyses of other crayfish samples prepared in the same manner, observed concentration values relative to aqueous standards are multiplied by this matrix factor, 1.4, to obtain true concentrations.

Figure 5 shows the relation between matrix factor and sensitivity for various sample types.

Another variable involved in matrix effect is the concentration of the sample matrix in the analytical solution on which the absorption measurements are made. That matrix factor if affected by matrix concentration is, of course, obvious; since, as the matrix concentration approaches zero as a limit, the matrix factor must approacn unity. However, the function is not linear with respect to its limit, as shown in Figure 6 for various matrix types and concentrations. The different ranges of matrix concentration shown here correspond to the typical ranges encountered in routine analyses of various samples types.

Another variable contributor to matrix effect is differences in aspiration rate between sample solutions and aqueous standards due to viscosity and density differences. Aspiration rates differing by a factor of two have been observed between aqueous standards and samples.

Aspiration rate differences have been eliminated through the use of a peristaltic pumping system to replace conventional air aspiration of solutions. The pump (Cole Parmer 7013) is driven by a synchronous motor at 60 rpm for a solution transfer rate of 3.6 ml/min. In addition to providing absolute control of solution uptake rate, oxidant flow rate is made an independent variable in optimizing flame conditions. In comparative studies of this system and conventional aspiration in the determination

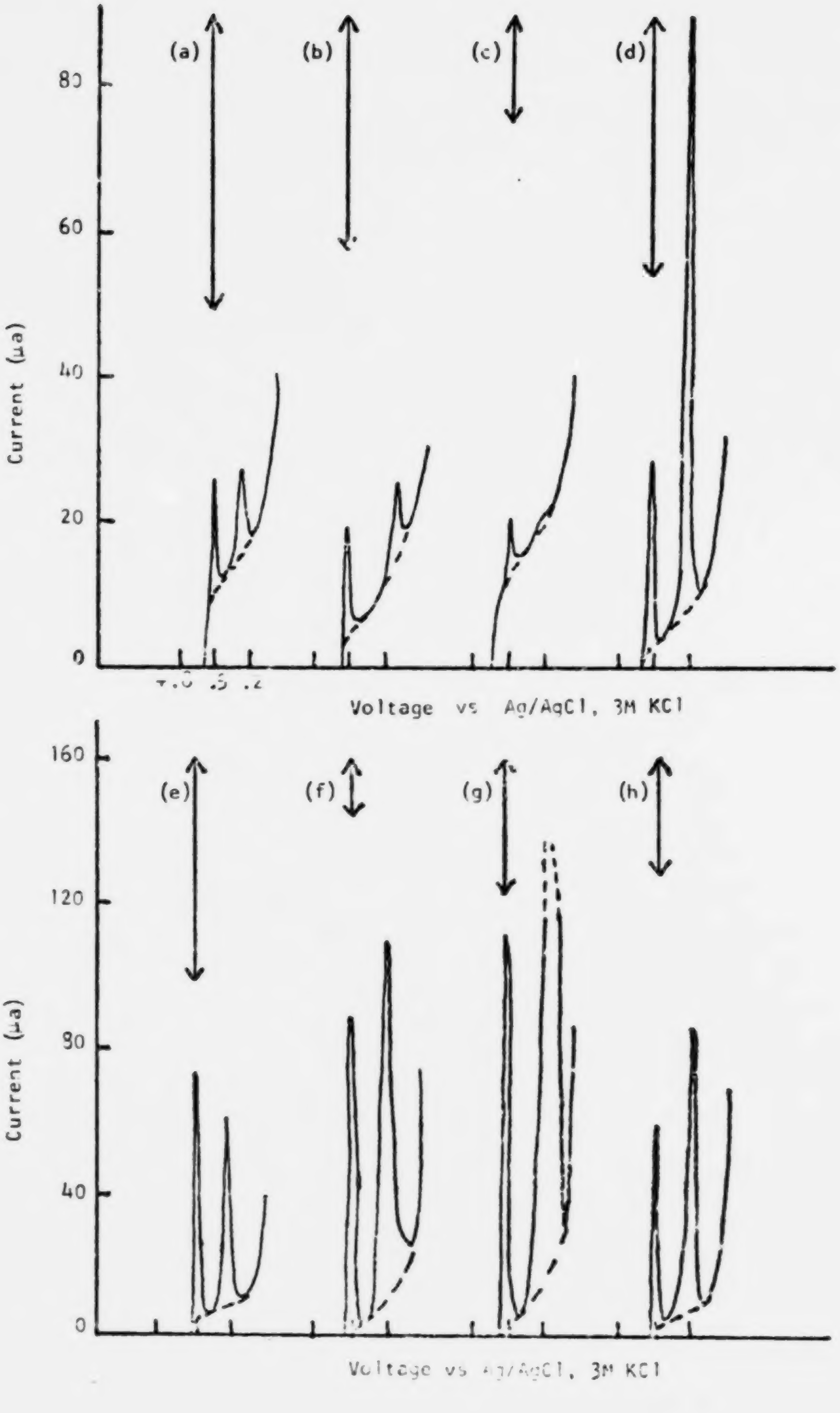


Figure 5. Typical anodic stripping voltammograms for various sample types: (a) lake water; (b) stream water; (c) sewage effluent; (d) earthworm tissue; (e) rat tibia; (f) rat blood; (g) rat muscle; (h) rat spleen. Length of arrow represents current increase for a standard addition of 10 ppb Pb.

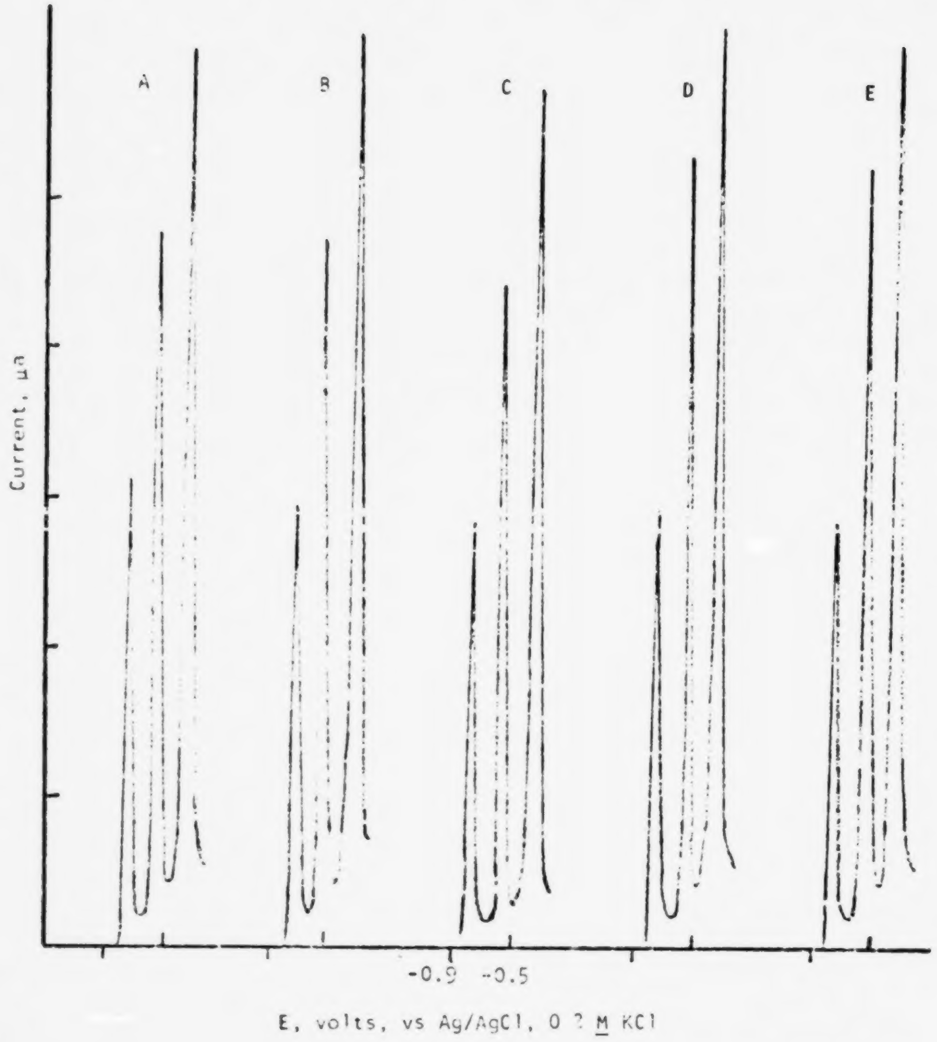


Figure 6. Typical curves for Cd, Pb and Cu in corn grain, using a Pyrolytic Graphite Electrode. Instrument is RC damped with time constant of 0.25 sec. Sweep rate of 2 V/min. All curves start at -0.9 V. Current per major division = 5 μa for A, B, D, E; 10 μa for C.

A, B. Sample pre-electrolyzed for 5 minutes.
C, Sample solution, 10 minutes pre-electrolysis.
D, E. Consecutive runs on same sample after a standard addition of 12.5 ppb Pb. Other conditions are same as for A, B.

of lead, improvement of analytical sensitivity between 30% and 50% has been observed.

2. Electrochemical Methods

a. Classical Polarography. The capabilities of ordinary 3-electrode classical polarography were examined for cadmium, thallium, and lead using the operational amplifier circuitry of the Enke-Brubaker type in the form of the Heath EA -19 Polarograph equipped with a Model EA -19 Chopper Stabilizer or a similar unit previously built by Dr. Hartley and co-workers in the School of Chemical Sciences. The latter unit is essentially the Enke-Brubaker circuit but uses G. A. Philbrick Associates Vacuum tube K-2X, K-2W, and K-2P operational amplifiers driven by a Philbrick R-100B Power Supply. The principal differences are a higher voltage limit of the Philbrick unit due to its ± 300 VDC supply compared to the Heath 150 VDC supply and a greater zero stability with lower current offset of the Philbrick operational amplifiers.

Typical polarograms of the three elements at concentrations just above the limit of detection are shown in Figure 4.

One of the principal difficulties in using 3-electrode polarography at the detection limit lies in determining an appropriate correction for the residual current. As shown in Figure 4, the individual drop profiles of the residual (capacitive) and the metal reduction (Faradaic) currents differ markedly. The pure capacitive current does decrease approximately as $t^{-1/3}$ following the increase in drop area while the Faradaic component increases as $t^{2/3}$. The net result for measurements where the residual current is exclusively capacitive and appreciable with respect to the Faradaic component is that normal residual current "correction" methods underestimate the magnitude of the Faradaic current. By measuring a series of diluted solutions of Pb^{+2} in 0.1 Molar KCl as electrolyte, it was established that a plot of i_d vs. [Pb] was not linear unless one measured the diffusion current as shown in Figure 4 at the top of the drop profile envelope (ignoring the transient "spikes") while estimating the residual current at the bottom of the envelope (Figure 4a).

The current transient "spikes" which appear at the top of the Faradaic wave at the end of each drop pose a special problem. These spikes appear to be characteristic of rapid response operational amplifier polarographs equipped with fast-pen recorders. Experiments with standard samples in these laboratories have shown that these transients are: (1) proportional to the diffusion current but must be ignored in its measurement (compared to Ilkovic Equation calculation); (2) uniquely dependent in occurrence and magnitude on the particular instrument, cell, electrode, and recorder used; and (3) dependent in magnitude and sign on the relationship of the electrode potential to the potential of the electrocapillary maximum. An additional characteristic of operational amplifier polarographs not ordinarily observed at higher concentrations is the effect of a prior Faradaic current on the stability behavior of a measurement. If one takes pains to scrupulously free a solution of oxygen and to use very pure electrolyte, the polarograms are characteristically more erratic showing more "spike" behavior than solutions of routine purity. We have attributed this behavior to the inability of ordinary operational amplifier circuits to handle mixed out-of-phase signals. As ordinarily used, the amplifiers tend to create the necessary "null" from the input and output (feedback) signals as if these were in phase. Where the actual input signal is composed of in-phase (Faradaic) and out-of-phase (capacitive) currents, the amplifier is never at true balance. This effect disappears if the input signal is made predominantly Faradaic by permitting a small, tolerable amount of oxygen to be present which creates a Faradaic signal where there would ordinarily be but capacitive currents (foot of the wave). Under these conditions, the drop profiles as well as the Faradaic currents for metals are more reproducible. These effects are seen by comparing Figure 4-B with its residual trace, Figure 4a-B as well as the Figure 4a-C pair. The (B) curves were obtained without complete deaeration which gave rise to the non-zero residual current shown in Figure 4a-B. Note that the drop profiles throughout are those associated with net Faradaic currents even at the foot of the wave. Figure 4a-C illustrates the difference between the nearly pure capacitive current of 4a compared to the changing profiles of 4-C. The straight lines in Figure 4 show how the residual current correction was obtained from the corresponding residual curve in Figure 4a.

The detection limits for Cd, Tl, and Pb (see Table 4) indicate that classical polarography will not be a heavily used method for these elements. Sample throughput for polarography is approximately 4/hour compared to 20-30/hour for atomic absorption spectroscopy. Since the latter method has a lower detection limit for these elements, polarography will be reserved primarily for higher level measurements where electrochemical techniques are needed to provide information about complexation, etc.

b. Anodic Stripping Voltammetry. This method has been the sole analytical method used in this laboratory for low level (parts per billion) concentrations of Pb, Cd, and Cu. It has been used for occasional measurements of Bi, Zn, and Tl. In theory, the method as commonly used with a mercury cathode ought to be applicable to any metal ion reducible to the metal within the decomposition range of the electrolyte. In practice the technique does not give good results unless the metal is soluble in mercury and does not form stable intermetallic compounds either with mercury or other co-deposited metals. Metals which deposit on mercury as insoluble films (e.g., Fe, Co, and Ni) do not yield reproducible anodic stripping results. Unless the electrode has a high hydrogen overpotential, the more reducing systems such as cadmium and zinc are not accessible in acidic solutions. Fortunately zinc, the most difficult metal to determine, has not been demanded in this project sufficiently to cause problems.

The only anodic stripping electrode routinely used to date has been the Mercury-Coated Graphite Electrode designed by Dr. Wayne Matson and sold by Environmental Science Associates, Cambridge, Massachusetts. In view of the fragility and cost of this electrode, the major electrochemical development work in this laboratory has been devoted to a search for alternative electrode systems. In total we have investigated six different electrodes in several forms and modes of use with limited but usable success in all. These are listed in Table 3 below. Each will be discussed in terms of reliability, ease of preparation, speed of analysis, sensitivity, and precision.

Wax-Impregnated Graphite Electrode. This electrode is purchasable from Environmental Science Associates at $98/electrode of which four are used in the ESA MASA-2014 Multiple Anodic Stripping Analyzer. The electrodes have undergone several evolutionary changes but basically consist of 4 1/2" x 1/4" low-density spectrographic grade graphite rods which have been paraffin-impregnated by a proprietary process probably similar to that first reported by Matson and Roe. The wax impregnation is removed for approximately one inch by light machining to expose a fresh carbon surface with its pores and cracks filled with wax. Such electrodes vary somewhat in projected surface area due to an indefinite boundary of the wax coating. Our estimate is that the average electrode has a gross surface area of 4.7 cm^2.

Table 3.

Voltammetric Stripping Electrode Systems

Substrate	Electrode	Form	Mass Transport Mode
1) Wax impregnated Graphite	Hg	Cylinder	N_2 Sparge
2) Pyrolytic Graphite	Hg	Disk	N_2 Sparge
3) Pyrolytic Graphite (a)	Hg	Disk	N_2 Sparge
4) Pyrolytic Graphite (b)	Hg	Disk	N_2 Sparge
5) Pyrolytic Graphite (c)	Hg	Disk	Electrode Rotation (600 RPM)
6) Platinum	Hg	Sphere	N_2 Sparge
7) Platinum	Hg	Wire Coil	N_2 Sparge or Rotation
8) Platinum	Hg	Disk	N_2 Sparge or Rotation
9) Pyrex	SnO_2	Cylinder	Rotation

(a) POCO graphite grade AXF -5Q1 (low density)

(b) POCO graphite grade AXF -50BG-1 (high density)
both (a) and (b) were obtained from Professor A. Beilby, Pomona College

(c) Pyrolytic Graphite - courtesy of Dr. T. Bednarski, Hercules Incorporated, Wilmington, Delaware.

The single common characteristic of all working electrodes used in electroanalytical measurements is a tendency to deteriorate from the moment they are made. An important part of the methodology which attaches to each electrode system is that which leads to restoration or replacement of the electrode. Of the many electrode systems which have been investigated, those which survive and are accepted in practice are those for which the replacement step is simple, reproducible, and reliable. For the same reasons, beginning investigations of new electrode systems are largely devoted to defining manifestations of deterioration, limits of acceptable treatment, and means of restoration. Such investigations usually reveal also the more important limitations of acceptable sample composition. In what follows each of the electrode systems listed in Table 3 is discussed in terms of its preparation, stability, and restoration.

Electrode deterioration is most commonly manifested in a decrease in the usable potential range and/or a decrease in the response sensitivity for one or all of the substances for which the electrode and method was usable. For the most common type of electrode, that which is used primarily as a cathode in the potential region negative (reducing) to common reference electrodes, deterioration is most quickly seen as a decrease in hydrogen overpotential. Fortunately a decrease in overpotential is not destructive in anodic stripping voltammetry until the decay is such as to interfere with the reduction process of the metal ion sought. For example, zinc cannot be reliably determined by ASV unless the hydrogen overpotential is high and diminished in importance by a high solution pH because zinc requires a large negative (-1.2v vs. SCE) plating potential. Of the "toxic" metals, cadmi cannot reliably be determined in acidic solution unless the electrode is nearly new while lead and copper can be determined in the same pH range with electrodes which are nearly exhausted.

The initial experience of this laboratory with the MASA-ESA unit and the MPGE (Mercury-Plated Graphite Electrode) was quite negative. After a frustrating period of six months, in which we had apparently destroyed four sets of four electrodes in attempts to achieve any sort of reproducibility, it was recognized that the root cause of the problem was the inherent self-destruction of these electrodes. The reason for wax impregnation is simply to fill the pores and cracks of the low-density graphite rods used. The paraffin wax itself does not become part of the active electrode surface. To the contrary, its presence on the graphite surface prevents the necessary adherent mercury plate from forming. The relatively low melting paraffin used is slightly water soluble and surface active toward carbon. Unless precautions are taken to minimize the possibility of paraffin transfer to the active electrode area surface, the electrode will exhibit rather rapid decay. Slight surfactant contamination can be removed by a brief rinse with acetone taking care that the rinse does not run from the wax-coated upper portion of the rod down across the electrode area. More stubborn cases require a better paraffin solvent such as ethylbenzene. In both treatments, success is shown by a satisfactory (> 1 volt) overvoltage after replating. If the surface is not so cleaned, the mercury coat is so non-adherent that it can be removed by the action of a nitrogen gas bubble during solution sparging. Even with solvent treatment, these electrodes will decay in sensitivity and overvoltage. A more drastic treatment consisting of a light abrasion with 320-400 grit carborundum paper followed by coarse paper towel polishing is required. Such treatments will restore electrodes to a useful state but only rarely and for unknown reasons can one rejuvenate an electrode to the original response as received from ESA.

The plating recipe given by Matson and the ESA-MASA instruction manual should be revised. The Matson procedure tends to overplate the surface yielding a relatively thick (> 30μ) mercury coating with consequent broadening of stripping peaks. The sole change should be in the time and manner of plating. Matson recommends plating at a potential of -0.4 volt vs. Ag/AgCl, 0.1 M KCl reference from 10 ml of a 7.8 x 10^{-5} $\underline{M}$ $HgCl_2$ solution, 0.0015 $\underline{M}$ in HNO_3 in two stages: 10 minutes with no stirring ("diffusion") plating followed by 20 minutes with nitrogen sparge stirring. We have found this to be irreproducible. We obtain much thinner plates and better electrode response by omitting the first stage and using just the 20-minute stirred solution step. It is believed that the first few minutes of plating are most important and that strict diffusion control of mass transfer cannot be maintained due to thermal convection and mechanical vibration problems.

The tolerance to solution components of this electrode is acceptable but definitely limited. Because the electrode mercury coat is so thin (> 10μ) and the quantity of mercury is so small (∿ 150μg), the electrode is quite sensitive to corrosive attack.

Moderate amounts (ppm) of oxidants such as Fe^{+3} (e.g., from blood, animal tissue, etc.) can destroy the mercury coating by oxidative attack unless the electrode is furnished cathodic protection by keeping it in the electrolysis circuit at all times. The effect is not instantaneous except for highly concentrated solutions which can destroy the electrode during the preliminary nitrogen sparge cycle where the electrode may not be connected into the circuit. For the same reason, it has proved impossible to achieve reproducible results from nitric acid solutions even at a pH of 3. Evidently the acid attack is severe even at the millimolar level.

The Wax-Impregnated Graphite Electrode has been used for the determination of lead, cadmium, and copper in a variety of samples. In terms of sample numbers the environmental waters (lake, stream, pond, or rain) comprise the largest group. This is because the instrument and method were dedicated exclusively to these samples for the past eighteen months. Only when it became apparent that one or more alternate electrode systems under development here would have requisite sensitivity was the equipment extended to other samples. Typical results are shown in Figure 5 . Note that the sensitivity arrow appears to vary with sample type. This is only partly true. Current practice in this laboratory is to replate electrodes daily. For reasons not yet clear, this results in a day-to-day variation in electrode sensitivity which is at least as great as the apparent sample variation in Figure 5 . Current experiments tend to indicate that the variation is exclusively in electrode preparation, not sample matrix.

The source and type of sample does affect the method but not in ways apparent from a single curve. Some matrices have proved surprisingly tractible. For example, bones (rat tibia) consistently give quite reproducible curves with flat baselines. When it is recalled that the matrix is a very difficult problem in atomic absorption, the results have been quite helpful. Water samples are troublesome. Sewage effluents tend to depress the electrode sensitivity due to adsorption of organics onto the electrode. Sensitivity can be restored at times with an acetone rinse but more often requires a thorough cleaning and replating. Dry-ashed samples give no trouble; wet-ashed samples destroyed by HNO_3 - H_2O_2 sometimes give erratic behavior which appears to result from incomplete removal of organic fragments.

The electrode does not tolerate HNO_3 even in moderate amounts. We have attempted to surmount the problem of samples in HNO_3 matrix by neutralization with disappointing results. The amount of base required for neutralization contributes an intolerable blank unless electrolytically purified.

Pyrolytic Graphite Substrate Electrodes. Three different samples of "pyrolytic" graphite have been obtained from the original investigators: two samples from Dr. A. Beilby of Pomona College in the form of 1/4-inch rods epoxy coated on the cylinder surface to produce a disk electrode and four fabricated disks 10 x 1 mm prepared by Dr. Theodore Bednarski of Hercules Incorporated. Because of their form, the Beilby electrodes were investigated using the MASA-2014 electrolysis unit. Initial results showed that these could be plated successfully using the standard procedure developed for the Matson electrodes. The most apparent difference between these and the wax-impregnated electrodes is the higher sensitivity of the former for lead, cadmium, or copper. If the intrinsic sensitivites for both types were the same, then measured sensitivity differences should be proportional to surface areas alone. Since the borderline between the wax layer and the exposed electrode surface of the Matson electrode is quite indeterminate, the comparison is not very accurate. Peak heights for anodic stripping analysis under identical conditions were compared. The projected surface area ratio is 12.5 while the comparable peak current ratio is 6.5. Thus, the Beilby electrodes appear to be about 1.9 times more sensitive on a per unit surface area basis. Since only two such electrodes were available and required the MASA electrolysis cell unit, further work has been postponed in favor of continuing output production with the complete four-cell unit using Matson electrodes. When time and personnel permit, these studies will be taken up again.

The more recently obtained pyrolytic graphite disks have been more extensively studied because their form permitted use in other electrolysis units. Dr. Bednarski indicates that he has had considerable success using these disks in a stationary horizontal mode using nitrogen sparge stirring. We have had similar success (almost quantitative agreement on electrodes prepared and used at Hercules before shipment to this laboratory). Plating treatment procedures are quite simple and appear to work without great detailed directions indicating the operation is quite tolerant of procedural changes. One detail that is markedly different from similar procedures for plating other substrates is the electrolysis potential. Mercury is reduced at or on a variety of substrates at potentials of 0 to -0.1 volt vs. SCE reference; pyrolytic graphite exhibits like behavior. Nevertheless, it is necessary to apply a potential of -1.0 volt in order to obtain a rapid plating that is adherent.

The Pyrolytic Graphite electrodes supplied by Dr. Bednarski were in the form of 1 cm x 1 mm disks. An electrode holder for stationary use was provided by Dr. Bednarski. It consists of a 4-inch length of Teflon rod which has been machined throughout most of its length to ca. 1 cm diameter with a 1 cm lower section somewhat larger (15 mm). The lower end of the rod is counterbored to a depth of 1 mm x 1 cm diameter to make a close fit with the graphite disks. The Teflon is bored lengthwise with a 1/8-inch hole for insertion of a few drops of mercury and a copper wire for electrical contact. The graphite disk is attached to a ring cut from vinyl electrical tape to have an outer diameter of 15 mm equal to the Teflon rod and an inner diameter of ca. 6.3 cm. Insertion of the disk followed by application of a slight vacuum on the back side of the disk through the rod created an effective seal of the tape.

A rotatable graphite disk electrode was fabricated in a fashion similar to that used for the platinum disk electrode. A decision made to include the vinyl tape under the epoxy cement was unfortunate: the vinyl tape proved to leak in time necessitating replacement but could not be replaced due to the over-coat of resin. The leakage was not discovered until after the experiments (which were erratic). As a result, all data are held in question and will be repeated in the near future.

The Bednarski electrode material itself is remarkable. Through a misunderstanding, this laboratory thought it was receiving pre-plated disks and proceeded to so use them with fair success. Lead, cadmium, and copper gave narrow, well-defined anodic stripping peaks. However, each voltammogram showed several satellite peaks which were not expected. Upon sending a tracing to Dr. Bednarski, we learned the electrodes had never been plated with mercury; the "satellite" peaks evidently arise from intermetallic compounds of the metals with the graphite. What is most surprising is that the unplated electrodes exhibited a very high hydrogen overvoltage at least as large as any mercury-plated electrode we have made. Upon proper plating, the overvoltage increased slightly and the satellite peaks disappeared.

Hercules and Dr. Bednarski to date have exhibited an understandable reticence about the mode of pre-treatment of these disks. In some manner the electrode surface is conditioned so as to favor rather strong bond-like attachment of mercury to the surface. Whether it is exact to state the connection as a mercury-carbon bond is as yet unknown to us. We do concur in the existence of a more-than-Van-Der-Waal adhesion as shown by the displaced anodic potential for mercury oxidation. This tends to substantiate a theory held by Hartley and co-workers that any substrate on which can be plated a reproducible adherence mercury film must undergo a definite chemical reaction with mercury for at least a monolayer at the substrate-mercury interface.

These electrodes give quite reproducible results. Figure 6 shows the response of a single electrode to duplicate 5-minute electrolysis followed by a 10-minute trial finally ending in a 5-minute electrolysis following a standard addition of 12.5 ppb lead. Experiments confirm that stripping peak currents are (a) proportional to lead concentration at fixed electrolysis time, or (b) to electrolysis time at fixed lead concentration. The latter is characteristic of electrochemical methods which measure concentration, not quantity of material.

Platinum Substrate Electrodes. This system has been the subject of investigations by Hartley and co-workers for the past six years. Present work has been devoted to understanding how to make and use these electrodes for analytical measurements.

In the previous report it was mentioned that platinum-based electrodes had a large disadvantage in a marked tendency to sequester deposited metals irreversibly. This effect can be overcome by a series of plating-stripping cycles in the same solution until "steady-state" anodic stripping peaks are obtained. In its worst form this effect required 6-10 cycles for each concentration of a metal for reproducible results. Our recent experience suggests that the effect can be minimized but perhaps not entirely eliminated. The following observations are pertinent:

1) The sequestering effect is always worse with newly-made electrodes.
2) Electrodes prepared by a mercury immersion technique have a higher hysteresis than those prepared by slow electrolysis.
3) Electrodes with thin films of mercury can be brought to a "steady-state" in 1-2 cycles whereas thick film electrodes prepared on the same wire may require 6-15 cycles.
4) Electrodes which have been stored for more than 24 hours always exhibit a marked hysteresis unless subjected to a "reversion" cycle before re-use.
5) Application of the reversion cycle also restores sensitivity to

an electrode .

6) Once made, an electrode improves with age in the sense that the substrate becomes easier to plate; prepared electrodes have a higher sensitivity and they exhibit less hysteresis.

Electrode preparation has been the subject of much investigation leading to the above observations. Our experience now suggests the following procedure leads to the most rapid development of a sensitive electrode:

1) Electrode metal should be fabricated before plating. Wires should be carefully sealed into maximum diameter soft glass tubing using good platinum-to-glass sealing techniques. Disks and other unsealed forms should be fastened to the appropriate glass electrode stem using a slow-drying formulation of Epoxy or Glyptal resins.

2) Pre-clean the electrode by thorough rinsing in acetone. If the electrode has been extensively handled, it may be necessary to use a benzene-chloroform-acetone sequence.

3) Immerse the electrode together with a platinum counter electrode into a 1 M $HClO_4$ solution and cathodize to rigorous hydrogen evolution using a convenient source of 1.5-6.0 volts. Effective cleaning of the electrode can be tested by opening the circuit, removing all gas bubbles from the electrode surface, and observing carefully as the circuit is restored with 1.5 volts. A clean electrode will turn a light gray, due to initiation of hydrogen bubbles, simultaneously at all points on its surface. There should be no preferential point of gas evolution. Cathodization can be permitted to continue until the next step.

4) Prepare a solution to contain approximately 0.16 mg mercury (as $HgCl_2$) in 0.15 $\underline{M}$ HNO_3 per square centimeter of electrode area. (A convenient solution is 10 ml of 7.8 x 10^{-5} $\underline{M}$ $HgCl_2$ in 0.15 $\underline{M}$ HNO_3 for a 1 cm^2 electrode). Use this solution in an electrolysis cell consisting of the electrode to be plated and a much larger platinum counter electrode. Connect the electrode as cathode to a convenient source of 1.5 volts and electrolyze without stirring for several hours--preferably overnight. This procedure requires that essentially all the available mercury be plated. Use of the dilute solution eliminates the necessity for monitoring the current.

5) At the completion of step 4, the electrode should have a grey to black coating. Apply a 6-volt dry cell briefly to the cell. If the plating has been successful, the electrode should immediately turn to a shiny metallic appearance similar to free mercury or to bright platinum upon application of the voltage and should remain so when the circuit is opened. If this does not occur, proceed to step 6.

6) Return to step 3 conditions. Continue cathodization at -1.5 volt with periodic tests using either -3 or -6 volts. When the electrode is clean, application of the higher test voltage will create the smooth and stable film desired. A critical test of the electrode at this point is to add a pool of pure mercury to the cell and observe the electrode as it is with -1.5 volts applied between it and the pool using the counter electrode as connection. Under these conditions, the electrode should evolve little or no hydrogen and none if the counter electrode is raised from the pool.

The Mercury Plated Platinum Wire Electrode. This electrode was fabricated for use in a rotated mode but was also used as a stationary electrode with nitrogen sparge mass transfer. A 4 cm length of B+S 24-gauge platinum wire was sealed in a 4-inch length of soft glass tubing then folded back and wrapped around the glass tubing snugly. The total exposed surface area was calculated to be 0.64 cm^2.

Synchron Model 610 (600 rpm) synchronous electric clock motors were used exclusively for all rotating electrodes with the exception of the SnO_2 electrode. The original 1/16-inch steel motor shafts were built up to the size required to fit the electrode glass tubing by nitric acid etching a short length of 1/8-inch o.d. brass tubing to be a snug force fit on the shaft, then further etching to be a close but not force fit into the glass tubing. An appropriate length of 28-gauge Nichrome or copper wire was hard- or soft-soldered to the terminal end of the brass sleeve. A few coil turns of the wire slightly extended provided a slight compression of the wire against the bottom of the glass tubing when the electrode was in place. The electrode was attached to the motor shaft with sealing wax. Electrical contact was made by amalgamating the upper end of the motor shaft and drilling the motor cover to hold a 21-gauge disposable hypodermic needle as a contact holder. A short length of 28-gauge amalgamated copper wire made light contact with the motor shaft. Electrical leads were either clipped directly to the copper wire or to the cutoff needle end. This arrangement is a most noise-free electrode contact for rotating electrodes and much superior to the more usual motor housing contact which is noisy due to bearing-shaft contact problems.

Typical anodic stripping results are shown in Figure 7. In contrast to other electrodes, this system showed a systematic increase in current with each repetition. The result in the precision estimates and sensitivities is not strictly comparable with other electrodes.

Rotating Platinum Disk Electrode. This electrode was fabricated similar to the rotated wire electrode. A 4-inch length of 1 mm Pyrex capillary tubing served as the electrode shaft. Attachment of the motor required that the motor shaft be ground and etched down to fit the capillary; sealing wax was again used to hold the electrode in place. A 0.5 cm disk of 28-gauge platinum sheet was cut using a cork borer. Attachment to the glass tubing was made by applying a light coating of epoxy resin cement to the capillary end and pressing the platinum disk on after allowing a brief period for the resin to set. Unless the amount of resin was kept small and allowed to set the excess resin tended to become squeezed into the capillary thus insulating the upper surface of the platinum from the necessary contact.

The electrode was plated as described for wire electrodes. Plating by immersion in mercury after scrupulous chemical and electrolytic cleaning was required to obtain a satisfactory plate. The excess of mercury acquired in this method was removed by several anodic stripping-cathodic deposition cycles in 1 $\underline{M}$ $HClO_4$.

Typical performance is shown in Figure 8. Note the sharper peaks obtained compared to the hanging mercury drop. The particular electrode used to obtain this curve was not created with a controlled film thickness.

The Hanging Mercury Drop Electrode. This electrode was investigated briefly as a point of reference for the other electrode systems. Inasmuch as this electrode has received extensive theoretical study by

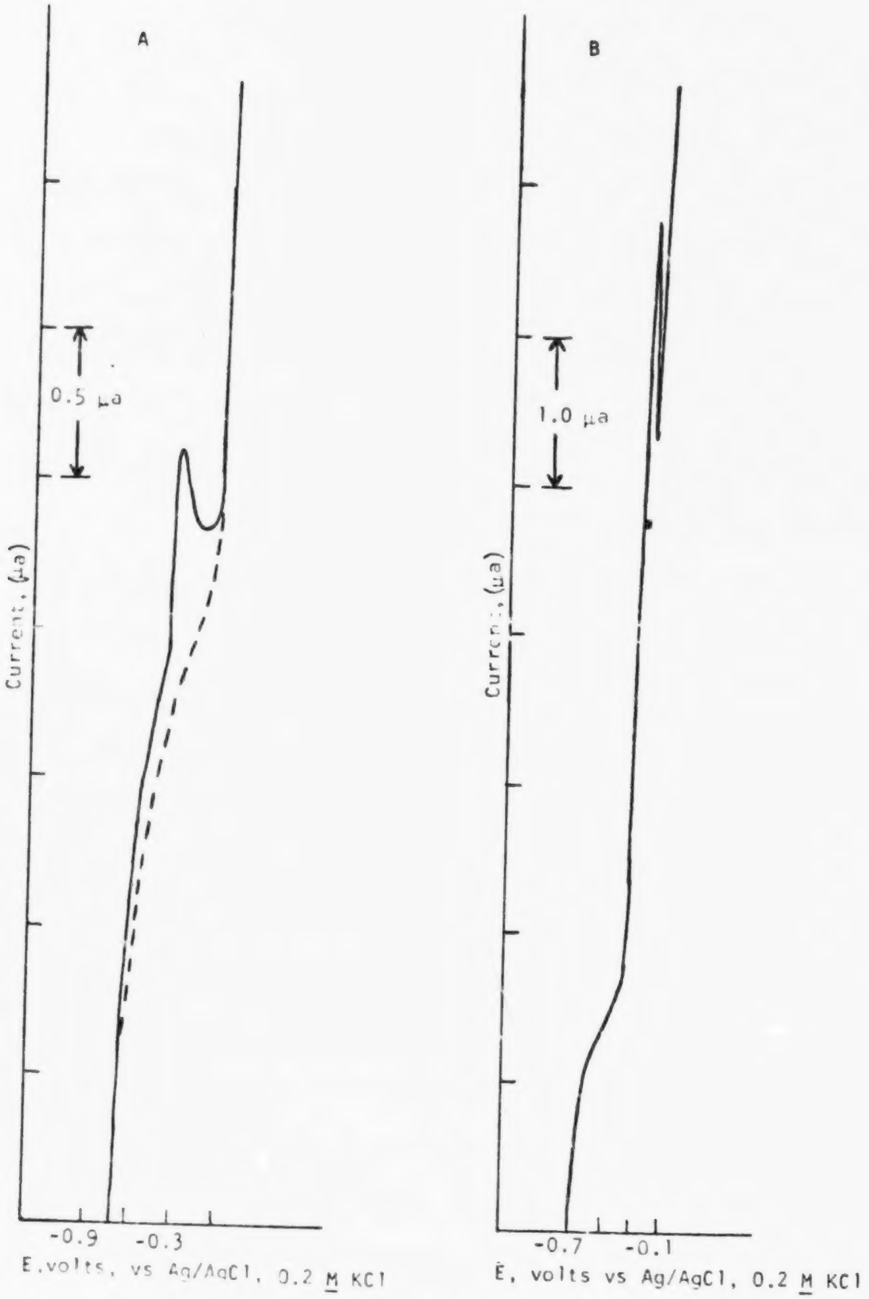


Figure 7. Typical curves for 20 ppb Pb (A) and Cu (B) in 0.06 M HNO_3 electrolyte with a Mercury Plated Rotating Platinum Wire Electrode. Instrument is RC damped with a time constant of 0.25 sec. Dashed line marks residual current curve in (A); dot marks the intersection with the residual current curve in (B).

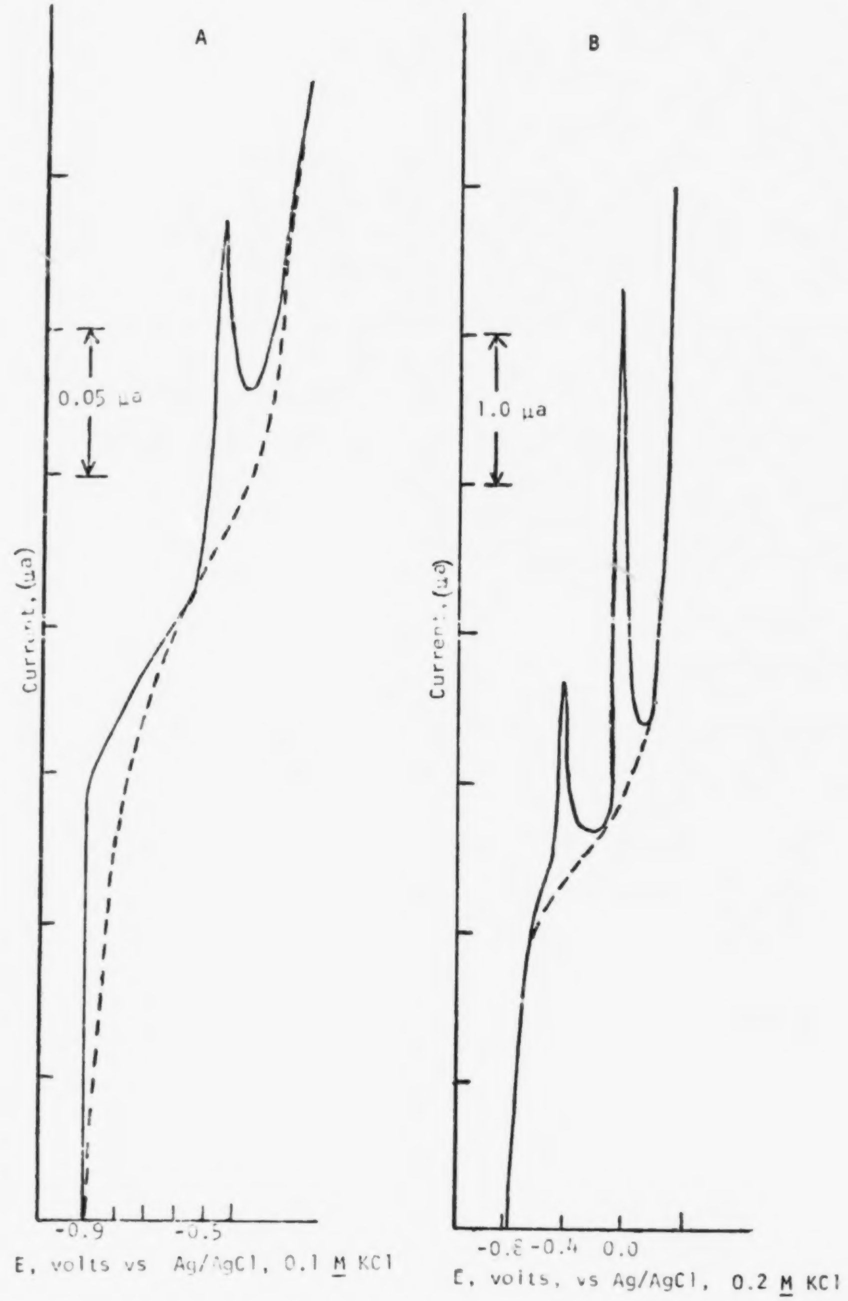


Figure 8. Typical curves for (A) 30 ppb PB in 0.1 M KCl with a Hanging Mercury Drop Electrode and (B) 20 ppb PB and Cu in 0.06 M HNO_3 with a Stationary Platinum Disc Electrode. Instrumental output is RC damped with a time constant of 0.25 sec. Dashed lines mark residual current curves.

deVries, Shain, and others, it was felt that this electrode would provide the most secure connection to theory.

The electrode proper consisted of a drop-hanger made of 6 mm soft glass tubing into which had been sealed a short length of 28-gauge platinum wire. After sealing, the wire and glass end were ground flush with 320 grit carborundum paper. The average electrode consisted of a mercury sphere of the collective volume equivalent to 3 consecutive drops from the D. M. E. which served as the in situ source of mercury in the cell. The calculated surface area was 0.19 cm^2.

A typical anodic stripping voltamogram is shown in Figure 8. Although satisfactory at the concentration level shown in Figure 8, the electrode characteristic of a diffusion controlled current decay beyond the peak resulted in a rather rapid merging of the peak into the background current. As a result, the limit of detection for this electrode was taken as that giving about 20 percent resolution of the peak from background (valley).

This electrode system proved difficult to use and not suitable for routine use. The physical requirements to provide for four electrodes (reference and compartment, counter and compartment, dropping mercury electrode, hanging mercury drop hanger), mercury drop transfer spoon, nitrogen sparge, sample inlet, sample drain, and nitrogen blanket inlet place excessive restrictions on minimum container size. The final result is that this electrode required a sample volume of 50 ml, higher by a factor of 5 over the next largest, thus increasing the limit of detection adversely.

Although the technician using this electrode had little difficulty, it is true that this electrode is delicately attached to the platinum contact and can be dislodged by sudden shock, thus destroying an experimental run. Since each run required 13 minutes for partial deaeration, plating and stripping the loss in sample throughput through electrode destruction would be significant.

Restoration of Electrodes. Old electrodes which have lost sensitivity and hydrogen overpotential or new electrodes which have failed to be satisfactorily plated can be recovered by a reversion treatment consisting of a brief anodization followed by replating. The procedure is:

1) Prepare a cell containing a mercury pool, a platinum counter electrode which can also serve as connector to the pool, and 10-15 ml of a millimolar solution of $HgCl_2$ in 0.1 $\underline{M}$ HNO_3 or $HClO_4$. Arrange the cell and test leads so that the electrode can be closely observed easily and cell polarity can be quickly reversed when necessary.

2) Connect the electrode as anode with a 1.5-volt source using either the Pt counter or Hg pool as cathode. Observe the electrode carefully. As electrolysis proceeds, the electrode will turn brown then black. As soon as the entire surface has turned black, reverse the polarity. Depending on the length of time the electrode remained an anode when black, it will undergo no change for some time (5-10 minutes). Eventually the electrode will turn light grey. Repeat this anodic-cathodic procedure several times, each time attempting to interupt the operation as soon as the electrode turns black or grey. It should be possible to reach the point where the electrode turns black all over immediately upon making it an anode and is restored to grey in less than 10 seconds of reverse treatment. At this point, apply -3 volts using the pool as anode. The electrode should become shiny and remain so.

Electrolysis Cells. Electrolysis cells were of two types: a simple 20 mm Pyrex test tube cut down to 90 mm length to fit the preformed cover of the MASA 2014 unit and an all-purpose cell fabricated of a 20 mm coarse porosity sintered glass filter funnel connected to a 1 mm Y-form 3-way stopcock and provided with a small side tubulation 5 mm from its top. The former cell was used exclusively for the Matson and Beilby electrodes with the ESA equipment. The latter was used with a dropping mercury electrode (DME), platinum wire in glass counter electrode, and a Ag/AgCl reference wrapped around the DME for polarography. All other electrodes were filled into a loose-fitting polyethylene snap-cap vial cover. Counter and reference electrodes were small glass compartments isolated by a porous Vycor plug, a Perley-type cracked glass, or a sealed-in asbestos fiber junction from the main body of solution. Nitrogen sparge was introduced at ca. 5 psig through the 3-way stopcock after de-oxygenation by passage through a vanadous sulfate wash tower. The second tubulation of the stopcock was connected to a trap and source of vacuum for aspiration of used samples. For those experiments where cell contamination due to prior usage was a possible problem, the cells were routinely leached overnight in concentrated Reagent Grade Nitric Acid before use.

In developing the data shown in Table 4, it was useful to express the dispersion estimate in two different ways so as to separate instrumental from procedural precision. The classical estimate of Standard Deviation, $\left(\frac{\Sigma(x-x)^2}{n-1}\right)^{\frac{1}{2}}$, strictly speaking should be applied only to data taken when all possible variables of the total method are allowed to operate. This applies to Column 4 of Table 4. A truncated dispersion estimate, Repeatability, $S.D._{repeat}$, can be defined for data obtained when some variables are not permitted to operate. This statistic has been held in disrepute due to misleading use of the term for self-serving reasons. Nevertheless, it is

Table 4.

Precision Estimates for Electrochemical Methods

	Element	Time Plate Strip Prepare (min)	Repeatability Std. Dev. %	Precision Std. Dev. %	Minimum Volume of Sample (ml)	Limiting Sensitivity (%/ng)	Limit of Detection (ng)	Limit of Detection ÷ Volume (ppb)
ASV								
Graphite	Pb	20	6	28	8	4.4	20	2.5
RPWE	Pb	10	12.3	3.9	7	2.4	41.4	5.9
	Cu	10	5	48	7	16.9	6	0.9
HMD	Pb	13	12	43.5	50	0.03	3800	76
PGE	Pb	10	12.4	2.5	7	7.6	13.1	1.9
SPDE	Pb	10	8	10	7	2.6	40	5.8
	Cu	10	0.3	6.7	7	114	0.87	0.12
Polarography	Pb	25	11	0.5	4	14.7 *	2720	680
	Cd	25	2.2	7.2	4	11.3 *	2120	530
	Tl	25	1.4	n.a.	4	13.7 *	2220	730

* %

useful and necessary when one wishes to compare two quantities which are dominated by a large variance estimate common to both but not germane to the point in question. In the present case, it is not useful to compare the total reproducibility of two methods when both carry a large but unknown variance component due to sampling, pre-treatment, and the like. In comparing methods, it would be useful to obtain precision estimates where only measurement variables operate. Most electrochemical measurement methods permit pure repetition because the act of measurement is essentially non-destructive. Atomic absorption cannot be put on the same basis strictly speaking, but a comparable statistic was generated by obtaining ten replicates of the act of atomic absorption measurement of a single standard aqueous solution.

Time (Column 2) was defined as that required to make a single measurement of a sample under routine conditions. The value excludes any sample preparation time and begins when the appropriate sample solution is introduced into the cell. The time does not include any standardization operations which are usually necessary for calculation of results. For all methods listed, the act of calibration consumes precisely as much time as a sample measurement. For a single measurement, the actual total operation time would be twice the figure given.

Precision (Column 4) was incomplete in that replicate original samples were not carried through sample pre-treatments. The variables of cell clean-out, washing, pipetting, electrolysis, etc., did operate. In all cases the number of replicates observed was four: precision estimates were obtained from ranges, S.D. $\hat{=} k_4[x_l - x_s]$.

Limiting Sensitivity (Column 6) is derived from the instrumental sensitivity and the Limit of Detection as defined in Table 5 . As it is defined it is a measure of the ability of a method to distinguish differences

Table 5.

Definition of Detection Limits and Sensitivity for Selected Analytical Methods

Method	Detection Limit(a)	Sensitivity(b)
1) Polarography	2 X drop oscillations	1% full scale(c)
2) ASV		
a) Hanging Mercury drop	20% peak-to-valley ratio	2 X S.D.$_{repeat}$ (renewed drop)(d)
b) Rotated Platinum	2 X S.D.$_{repeat}$	μa/ng.
c) Stationary Platinum	2 X S.D.$_{repeat}$	μa/ng.
d) Pyrolytic Graphite Electrode	2 X S.D.$_{repeat}$	μa/ng.
e) Wax-impregnated	2 X S.D.$_{repeat}$	μa/ng.
f) Atomic Absorption	1% Abs.	Abs/μg(e)

(a) Concentration which produces readout equal to the stated definition.

(b) Change in readout value per unit change in concentration except where indicated.

(c) Sensitivity is defined as the concentration change necessary to create a 1% full scale change in current.

(d) Defined as the concentration change which will change the readout value by twice the repeatability expressed as standard deviation.

(e) The change in absorbance per unit change in concentration.

between samples at the Detection Limit level or slightly above; it is a worst case figure. Alternatively, it expresses the percent of the detection limit signal that would be observed if the solution concentration were changed by 1 ng. A few examples will illustrate the use of the data in Table 4. Entry 1, the wax-impregnated graphite electrode, is seen to be relatively imprecise, S.D. = 28% (Column 4), but moderately sensitive requiring 8 ml (Column 5) of a 2.5 ppb (Column 8) solution of lead to give a peak current which is twice the repeatability. What is the least difference between samples each measured once which would be significant? For paired measurements having a 28% precision, the difference must be at least 1.41x S.D. or 39%. Column 6, the Limiting Sensitivity, shows a 4.4% change in signal per nanogram of lead added at the 2.5 ppb level. Hence, the two solutions would have to differ by 39 ÷ 4.4 or about 9 ng. A 9 ng change in 8 ml of solution corresponds to 1.1 ppb or a 44% difference between two solutions.

Table 6 shows an interesting comparison of four typical electrodes studies: Rotated Platinum Wire Electrode (RPWE), Hanging Mercury Drop (HMD), Pyrolytic Graphite Electrode (PGE), and Stationary Platinum Disk Electrode (SPDE). The factors governing peak currents (ip) to experimental variables are given by:

$$ip = k_P k_S [M] A t_{el} v$$

where k_P = mass transfer rate for metal deposition, k_S = mass transfer rate for stripping cycle, [M] concentration of deposited metal in the bulk of solution, t_{el} = electrolysis time, v = sweep rate for stripping cycle.

All electrodes were observed using the same 2 volt/minute sweep rate. Estimated surface areas from projected geometrical areas of the substrates are given in Column 2. Values in Column 3 are obtained by normalizing the observed peak currents to values expected for a 20 ppb solution of lead

Table 6.

Collection Efficiencies for Anodic Stripping Electrode

Electrode	Area (cm^2)	(μa)
RPWE	.64	1.408
HMD	.19	0.092
PGE	.31	13.2
SPDE	.20	7.35

electrolyzed for 5 minutes assuming linearity between concentration or time, surface area, and peak height. The values in Column 3 are in the expected order: HMD, RPWE, SPDE, and PGE. What was not expected was the 144-fold increase between PGE and HMD. The HMD value is depressed because the delicate nature of this electrode does not permit any effective stirring, hence the result is nearly that for a diffusion-controlled electrolysis. Both PGE and SPDE measurements were done with nitrogen sparge stirring. This is thought to be ineffective compared to electrode rotation in the >600 rpm convective region. Since both "stationary" electrodes were tilted some 15-20° from the horizontal to prevent nitrogen bubble buildup, it may be that the rather rapid lateral bubble passage across the face of these electrodes is quite effective. We will continue to examine these problems by comparing these electrodes in quiet, nitrogen sparge stirred and rotated modes.

c. Cathodic Stripping Coulometry. A method has been developed that has several advantages over previously published cathodic stripping methods for lead. Quantitative deposition of the lead eliminates the necessity for an analytical working curve and permits the highest possible sensitivity. The use of a SnO_2 semiconductor electrode also permits higher sensitivity due to its inherently low residual current. The SnO_2 electrode has lower double-layer capacitance, higher oxygen overvoltage, and fewer surface Faradaic reactions than other solid electrodes.

The cathodic stripping of lead involves two steps: a preconcentration step in which the ion in solution is quantitatively oxidized and deposited on the surface of a SnO_2 semiconductor electrode, followed by a stripping step in which the amount of PbO_2 deposit is determined coulometrically, using either a constant current or linear voltage scan technique.

Theoretical Considerations. To deposit PbO_2 quantitatively from dilute solutions of Pb^{+2}, it is necessary that a soluble intermediate not be involved in the oxidation mechanism. Experiments with the rotating ring-disc electrode indicated no such intermediate and thus directly substantiated previously proposed mechanisms involving an adsorbed intermediate. The PbO_2 deposition takes place in two distinct stages, nucleation followed by crystal growth. Potentiostatic studies of the kinetics for nucleation and growth at high overpotential indicate that nucleation takes place randomly on the electrode surface, followed by three-dimensional growth. Scanning electron microscope observations of the PbO_2 deposits substantiate a non-preferred growth process at higher overpotentials. Hemispherically-shaped crystallites of random size and distribution are observed.

Voltammetric stripping of the PbO_2 from the SnO_2 electrode indicates two distinct current peaks, which are attributed to PbO_2 of two different activities. Potentiostatic deposition studies indicate that a sharp cathodic current peak near the reversible potential corresponds to the dissolution of PbO_2 of normal activity from the bulk of the crystal and that a broader, more cathodic peak corresponds to the dissolution of a fractional monolayer of PbO_2 directly bonded to the SnO_2 surface.

Practical Considerations. Quantitative deposition of the lead is achieved within relatively short times by using a cell with a large electrode surface area to solution volume ratio. A cylindrical rotating electrode is used to optimize the cell geometry, giving a deposition half-time of 1.3 minutes with a 15 cm^2 electrode area, 2.5 ml solution volume, and 63 radians/sec rotational speed.

Two types of media have been investigated: 10^{-3} to 1M HNO_3 and 0.2M acetate buffer, pH 5.2. In the more concentrated nitric acid, depostion is initially determined completely by the rate of nucleation and growth. In HNO_3 less than about 10^{-2}M, the deposition is completely mass transport controlled. In acetate medium, the rate of deposition is also determined predominantly by mass transport. However, the PbO_2 is not completely dissolved during the reduction step, requiring an additional reduction in nitric acid before each determination in acetate.

Several potential interferences have been studied. Chloride and water do not chemically reduce the PbO_2 deposit within the pH range used. Phosphate causes the PbO_2 to be deposited predominantly on the SnO_2 surface. A neutral surfactant does not interfere with quantitative deposition.

The present limit of detection is approximately 5×10^{-8}M Pb^{+2}. At this level, the PbO_2 exists predominantly in the form of material directly bonded to the substrate.

Experimental Details. An attempt was made to detect soluble intermediates in the oxidation and reduction processes using a rotating ring-disc electrode. As the Pb^{+2} was oxidized at the SnO_2 disc in 1M HNO_3, the ring was potentiostated at a cathodic potential to reduce the possible intermediate. This was repeated for the reduction of PbO_2. No reducible intermediate was detected in either case. This experiment does not preclude the presence of a soluble intermediate. There is still the possibility of an unstable soluble intermediate decomposing too rapidly to be detected at the ring electrode. If such is the case, the decomposition reaction must have a rate constant greater than 3×10^{2} sec^{-1} assuming first-order kinetics.

Nucleation and Crystal Growth. Fleischmann, *et al.*, (1958, 1959, 1960, and 1963) have studied the deposition of PbO_2 on Pt from nitric acid and acetate media by measuring current-time curves. They conclude that PbO_2 nucleates according to the first-order law $N = N_\circ[1-\exp(-At)]$ where A is the potential-dependent nucleation rate constant and $N_\circ$ is the maximum number of nuclei possible per unit area at a given potential.

He also concludes that the individual centers grow according to a simple three-dimensional model in the form of a hemisphere. The current-time relationship for a single center has the form $i_3 = B_3t^2$ where B_3 is proportional to k^3, k being the potential dependent electrochemical rate constant for unit surface area.

Combination of the linear nucleation and the three-dimensional growth laws gives the overall measured current density $i = \frac{AB_3N_\circ t^3}{3}$.

The experimental current-time curves do not fit this relationship exactly, leading Fleischmann to assume an induction period, $t_\circ$, preceding nucleation, and to modify the equation to $i = \frac{AB_3N_\circ(t-t_\circ)^3}{3}$.

By pre-forming $N_\circ$ nuclei at a higher overpotential, the current-time relation reduces to $i = BN_\circ t^2$. Fleischmann (1959) has measured the parameters A and $BN_\circ$ and $t_\circ$ as functions of potential, pH, and lead concentration.

The deposition of PbO_2 on the SnO_2 surface obeys the same laws of nucleation and growth as Fleischmann observed on Pt. Even the apparent induction period was observed. The expected hemispherically-shaped crystals were observed using the scanning electron microscope. Data for this section of the investigation are not extensive, but are sufficient to verify that the deposition of PbO_2 conforms to the same nucleation and growth laws on SnO_2 as have been observed on Pt.

Constant Potential Deposition Followed by Linear Sweep Voltammetry. When the PbO_2 deposit is stripped by linear sweep voltammetry two current peaks are observed, a sharp reversible peak and a broad, more cathodic peak. We have attributed this broad secondary current peak to the reduction of the PbO_2 directly bound to the SnO_2 surface. Observed trends tend to verify this assumption. The conditions of forming the PbO_2 deposit greatly affect the relative sizes of the reversible and secondary peaks.

General Trends. PbO_2 has been stripped after deposition at a high overpotential for progressively longer times. For relatively short deposition times, only the secondary stripping peak is observed. At high deposition overpotentials, the size of the stripping peak increases with deposition time and attains a maximum size. At longer times the reversible peak finally appears and grows with further increase in deposition time. The secondary peak size increases further as the reversible peak increases in size. Such behavior is consistent with the previously-observed nucleation and growth laws, assuming the secondary reduction peak is due to PbO_2 directly bound to SnO_2.

At high overpotential, nucleation proceeds to form the maximum number of nuclei N_o before significant growth occurs. This process corresponds to the initial growth of the secondary peak at short deposition times. After the maximum number of nuclei is formed, the nuclei begin to grow, giving rise to a levelling off of the secondary peak and initiation of a reversible reduction peak. As the growth of PbO_2 progresses, the amount of PbO_2 bound directly to SnO_2 increases due to the base of the hemispherically-shaped crystal increasing in size. This corresponds to the further increase in the secondary peak size.

At low overpotential, the rate of nucleation is much slower, the reversible and secondary peaks both occur at short deposition times, both increasing with longer times. This is expected since growth proceeds before nucleation is completed.

Quantitative Correlation. The strongest evidence to support the assumption that the secondary reduction peak is due to a PbO_2 monolayer bound to SnO_2 is the fact that the quantity of this deposit formed as a function of deposition time conforms to the nucleation law: $2.303 \log [1 - \frac{N}{N_o}] = -At$.

If we assume that at a constant overpotential that

(1) the nuclei are of uniform size (or average size)

(2) the nuclei do not increase in size with time, i.e. the two-dimensional growth rate is negligible,

then the ratio $\frac{N}{N_\circ} = \frac{Q}{Q_\circ}$ where Q and $Q_\circ$ are the charges per unit area required to reduce the nuclei formed after time t and the maximum charge, respectively. The previous equation can be rewritten as $2.303 \log [1 - \frac{Q}{Q_\circ}] = -At$. Experimentally, a plot of $\log [1 - \frac{Q}{Q_\circ}]$ vs. t should be linear and have a slope $-A/2.303$. A relatively high overpotential (+0.902V vs. PbO_2/Pb^{+2}) was used in this study so that the nuclei did not grow appreciably before the maximum number was formed. High lead ion concentration (0.25 M $Pb(NO_3)_2$) was used to minimize oxidation of water at the electrode. The nitric acid medium (1 M HNO_3) was chosen due to the good separation of reversible and secondary peaks.

Figure 9 shows the series of linear sweep stripping curves obtained for deposition times from 0.5 to 10 ms. Deposition was repeated three times at each time to check reproducibility. Notice that growth is proceeding at longer deposition times. The secondary peak area had to be estimated by extrapolation at longer times.

Figure 10 is plot of secondary peak charge density vs. the deposition time. A maximum charge density $Q_\circ/A = 8.3\ \mu c/cm^2$ is estimated from this curve at longer times. This corresponds to approximately 3% of a monolayer coverage, the monolayer charge density for PbO_2 being 263 $\mu c/cm^2$.

Figure 11, a plot of $\log (1 - Q/Q_\circ)$ vs. t, is in fact linear with zero intercept. The zero intercept indicates a very short induction period at this high overpotential. The induction period, if it exists, is less than 50μS. Fleischmann (Fleischmann, et. al., 1958) has verified a logarithmic dependence of the induction period on overpotential in acetate media.

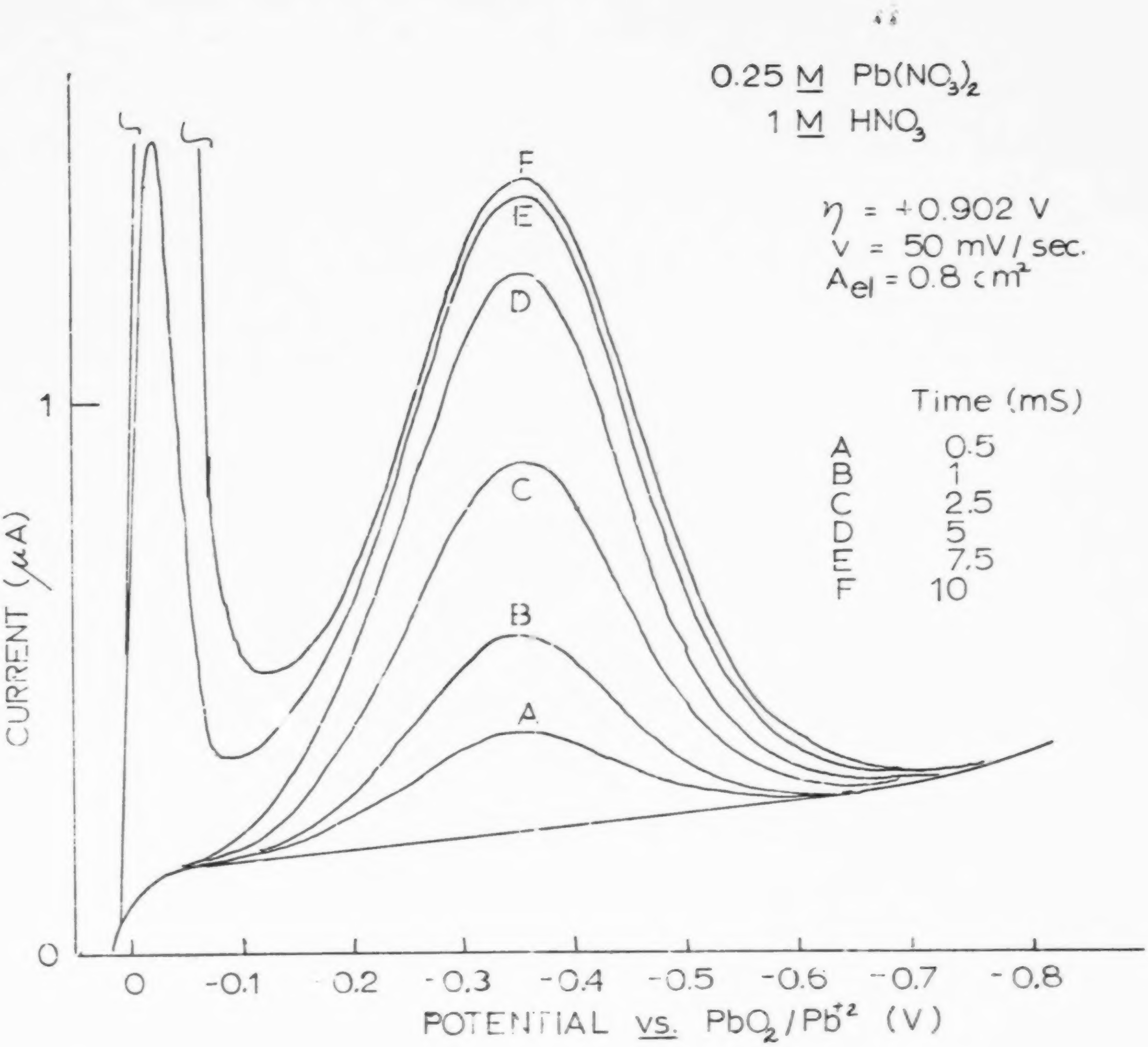


Figure 9. Linear Potential Sweep Stripping of Lead Dioxide Deposited at Constant Overvoltage.

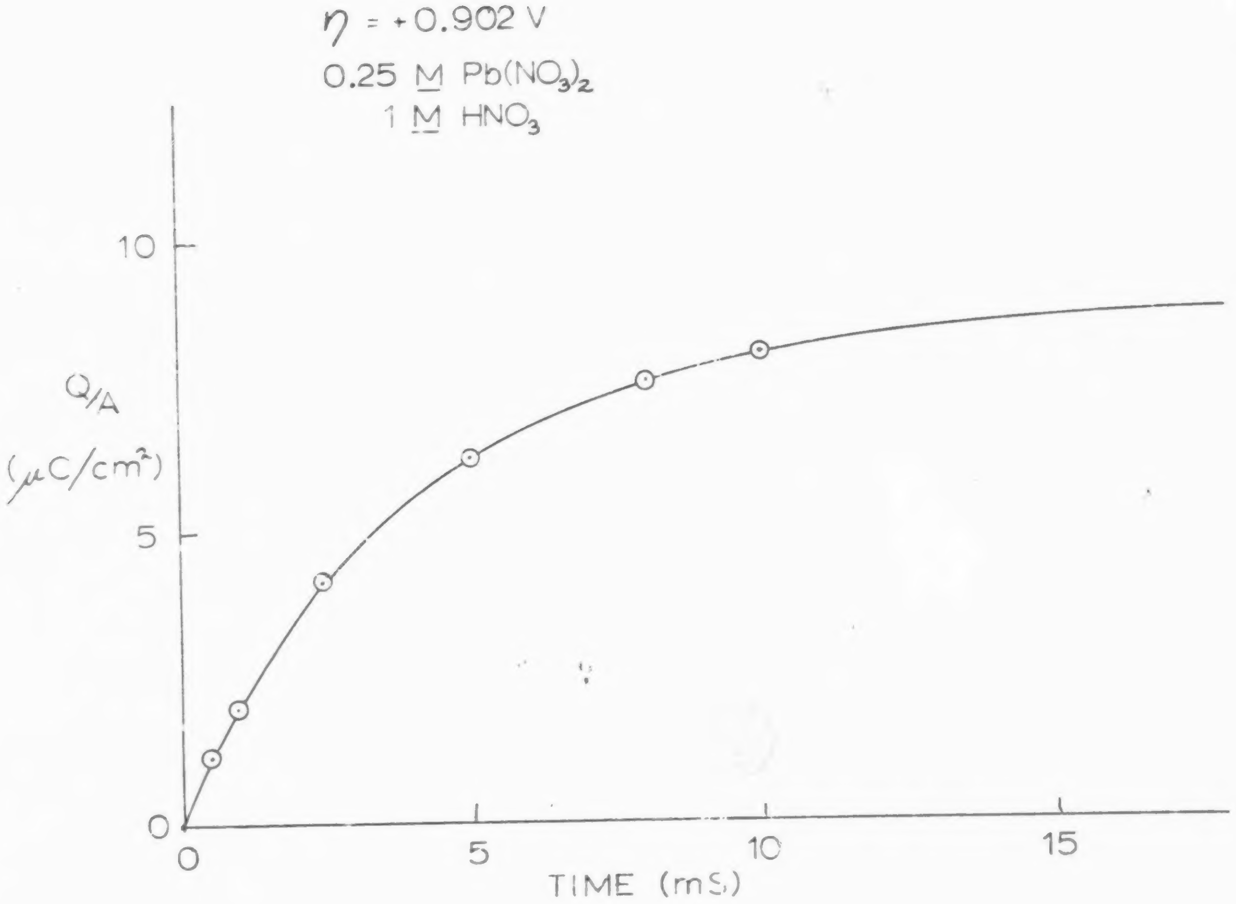


Figure 10. Secondary Current Peak Charge Density vs. Deposition Time at Constant Overvoltage.

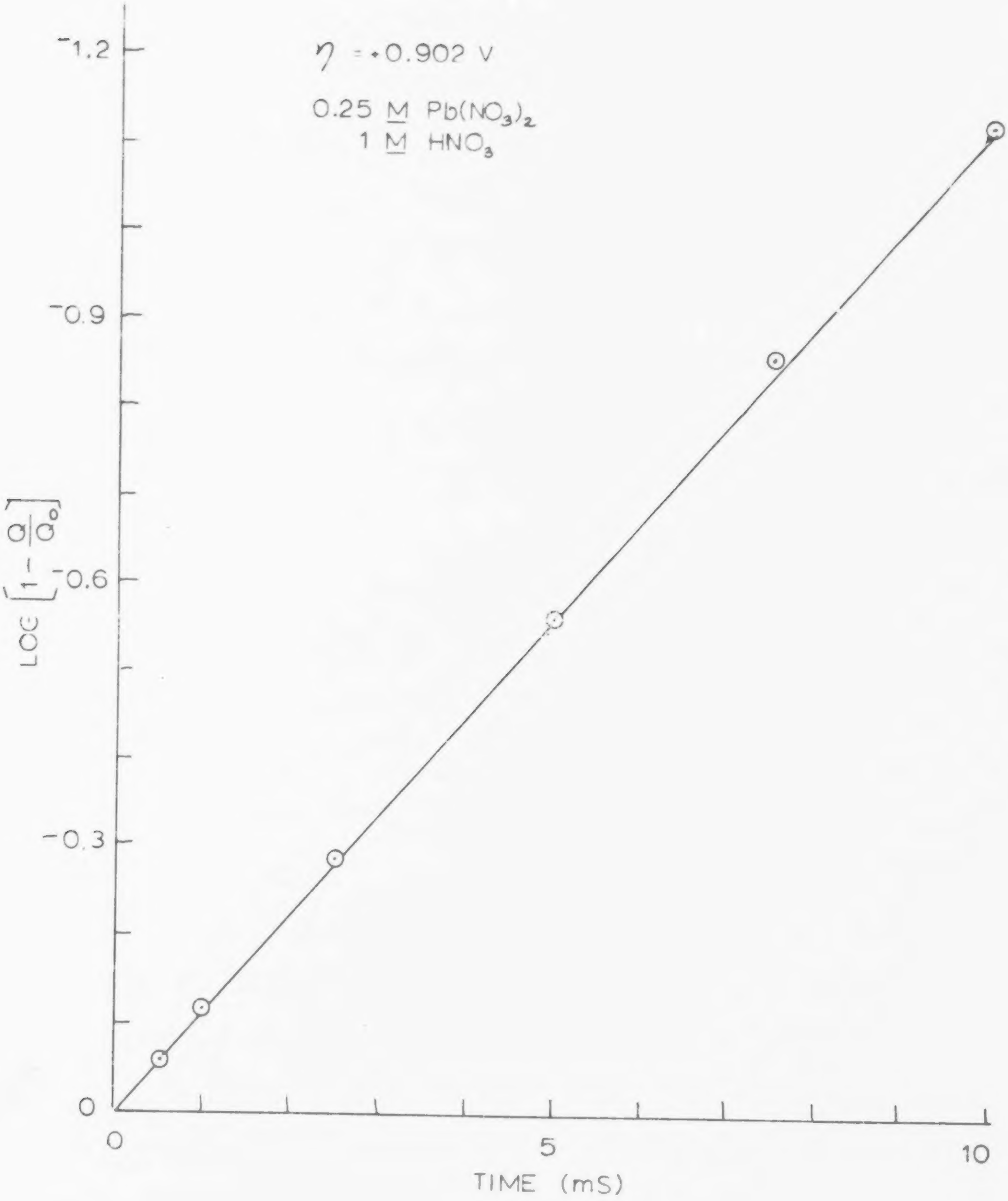


Figure 11. Logarithmic Plot for First-Order Nucleation Kinetics.

At the high overpotential used in our experiment we would expect the induction period to be quite short. The nucleation rate constant A equals 208 sec^{-1} as determined from the slope of log $[1 - Q/Q_2]$ vs. t agrees well with an extrapolated value of approximately 100 obtained from a graph of log A vs. $1/\eta^2$ for 0.25 M $Pb(ClO_4)_2$ in 0.1 M $HClO_4$ (Fleischmann, et. al., 1959, Figure 12), for which the highest overpotential used is $\sim + 0.25$V.

The general behavior observed for the relative quantities of charge involved in the reduction of the two peaks suggests that further quantitative information might be obtained to support our hypothesis. The hypothetical relationship between the two peak charges can be derived from a simple model requiring a few assumptions:

(1) All nuclei are of the same age, so that all crystals are identical.

(2) Crystals grow in three dimensions as hemispheres from a point nucleus.

Using these assumptions, one can calculate the relation between hemisphere volume and surface layer volume as a function of radius. A plot of log $[Q_{\text{film}}/Q_{\text{hemisphere}}]$ vs. log $[Q_{\text{hemisphere}}]$ should have slope - 1/3.

Experimentally, the nuclei should be preformed to assure that all nuclei are the same age and that the growing cystallites are the same size. In this particular experiment the double pulse method was not used, but rather high overpotential (+.47V) was applied. It was initially assumed that the rate of nucleation is instantaneous compared to the crystal growth rate, so that the maximum number of nuclei is formed before significant growth occurs. The solution is again 0.25 M $Pb(NO_3)_2$ in 1 M HNO_3. The total amount of deposit ranged from 11 to 4640 $\mu c/cm^2$, or from 0.05 to 14 times the monolayer charge density. Typical cathodic stripping curves are presented in Charts 1a-1d. The reversible peak overlaps the secondary peak at high charge densities, necessitating the extrapolation of the secondary peak by assuming it to be symmetrical. Also at higher charge densities the reversible peak currents were so large compared to the secondary peak that the current sensitivity had to be switched at a potential between the two peaks.

Figure 12 is a plot of [Q secondary/Q reversible] vs. Q reversible. The plot of log (Q_2/Q_1) vs. log Q_1 has a slope of -0.91 instead of the expected - 1/3, indicating that Q_2 is essentially constant. In fact Q_2 only increases by a factor of three for an increase in the ratio of Q_1/Q_2 of approximately 300.

The discrepancy between experimental results and prediction can be rationalized in several ways. Perhaps during the initial stages of growth the growth geometry is predominantly one-dimensional, rather than the three-dimensional hemispherical growth observed during later stages of growth. Fleischmann (Fleischmann, et. al., 1960) claims that "in the initial stages of growth of α-PbO_2 from acetate solutions, the deposit is formed predominantly in the [002] one-dimensional orientation at low and intermediate potentials". He further states that "the deposit is formed without preferred orientation at high overpotentials and also from nitrate solutions". These statements tend to discount the possibility of one-dimensional growth, but on the strength of our own stripping current measurements and reasonable confidence in our model and associated assumptions one would tend to conclude otherwise.

Design of Electrolysis Cell. Quantitative deposition of the lead is achieved within relatively short times by using a cell with a large ratio of surface area to solution volume. The cell is pictured in Figure 13, and consists of a cylindrical rotating SnO_2 electrode in a tubular cell wall with sidearm reference and counter electrode compartments isolated by porous Vycor junctions. The electrode is rotated at 600 rpm with a Sargent Synchronous Rotator and electrical contact is made by a mercury pool. Electrode area is approximately 15 cm^2, and solution volume is 2.5 ml.

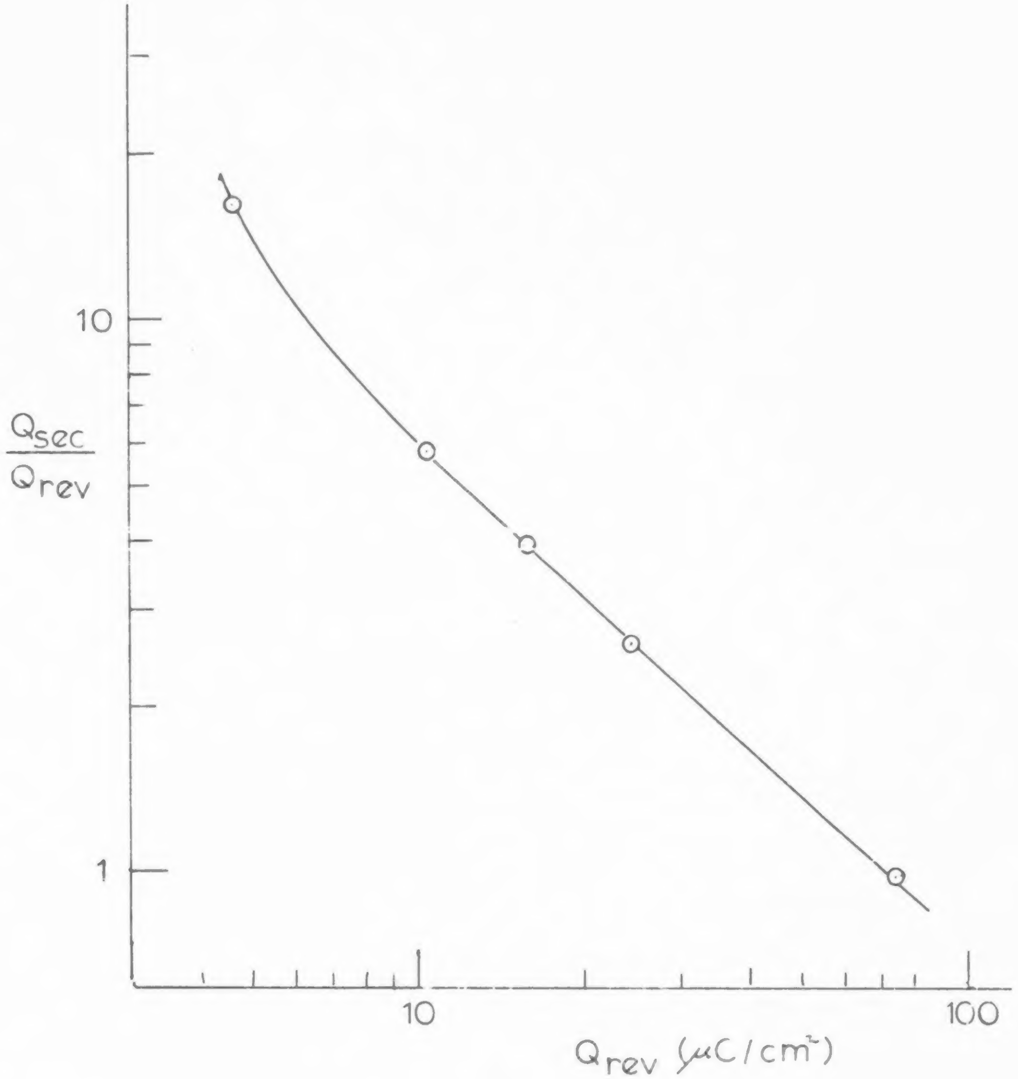


Figure 12. Relationship Between Reversible and Secondary Peak Charge Densities.

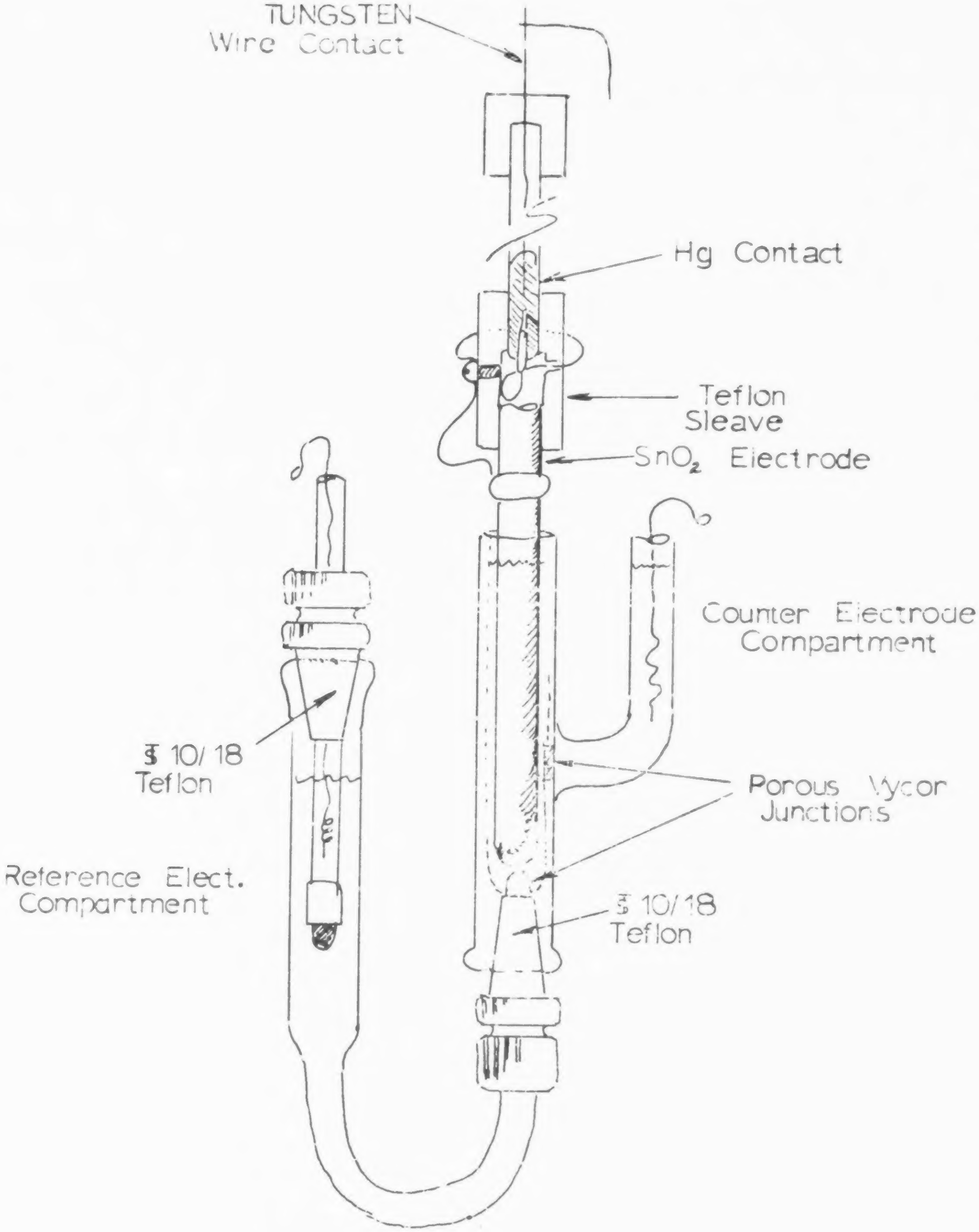

TUNGSTEN
Wire Contact
Hg Contact
Teflon
Sleave
SnO_2 Electrode
Counter Electrode
Compartment
Porous Vycor
Junctions
$ 10/18
Teflon
Reference Elect.
Compartment
$ 10/18
Teflon

Figure 13. Cylindrical Tin Oxide Electrode and Cell Assembly

The diffusion layer thickness is 2.2×10^{-3} cm, and the electrolysis half-time is 1.3 minutes. Within 6.7 half-times, or 8.7 minutes, 99% of the lead would be deposited if the oxidation is mass transport controlled.

Effect of pH on Deposition Rate. An experiment was designed to determine the effect of pH on the overall rate of deposition from nitrate media. The hydrogen ion concentration was varied from 10^{-3} to 1 M, and the nitrate concentration was maintained at 1 M with $NaNO_3$. The electrolysis potential varied with pH to maintain a constant overpotential vs. the PbO_2/Pb^{+2} equilibrium potential: $4H^+ + PbO_2 + 2\bar{e} \rightleftarrows Pb^{+2} + 2H_2O$; $E = E° - \frac{RT}{2F} \ln \frac{a_{Pb^{+2}}}{a^4_{H^+}}$; $\frac{\delta\,(E - E°)}{\delta pH} = \frac{0.118V}{\log a_{H^+}}$. The results are summarized in Figure 14. At high acid concentration the nucleation and growth are so slow that only a small fraction is deposited even after 20 minutes. The deposition is controlled completely by heterogeneous kinetics. At lower acid concentrations, 10^{-3} M for example, the deposition rate is still initially controlled by the nucleation rate for the overpotential used; but after about 5 minutes the rate is completely mass transport controlled. Figure 15 shows that with 10^{-2} M acid the deposition can be completely mass transport controlled at higher overpotentials, for which the nucleation and crystal growth rates are faster.

The conclusion to be drawn is the pH is a dominant factor controlling the deposition rate and must be maintained above about pH 2 to achieve mass transport controlled deposition from 10^{-6} M Pb^{+2} solutions. A higher pH limit may be required for lower concentrations of lead.

Study of Potential Interferences. Chloride is a common constituent of natural samples, and HCl is widely used to digest and extract solid samples. Therefore it is of prime importance to evaluate the effect, if any, chloride has on this technique. One of the predictable effects would be to

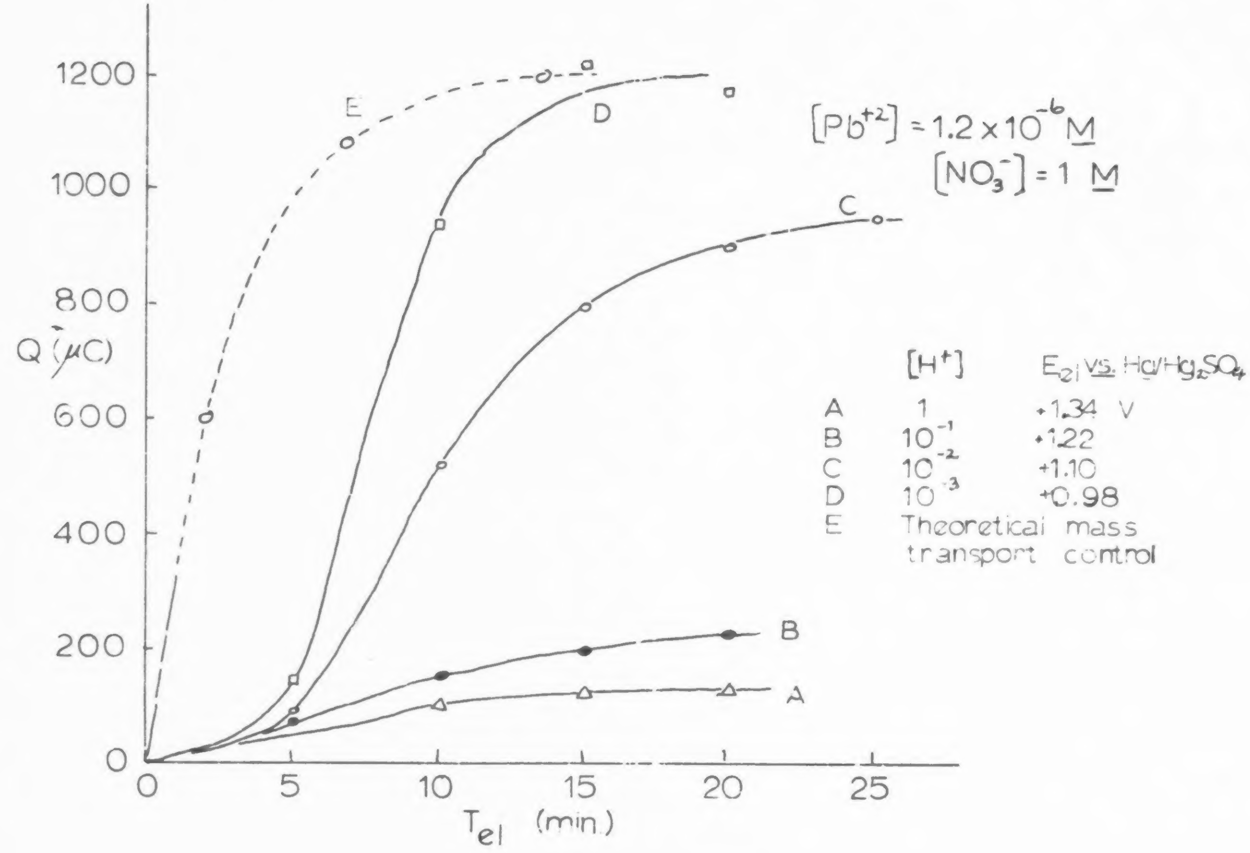


Figure 14. Lead Dioxide Deposition Rate as Function of pH.

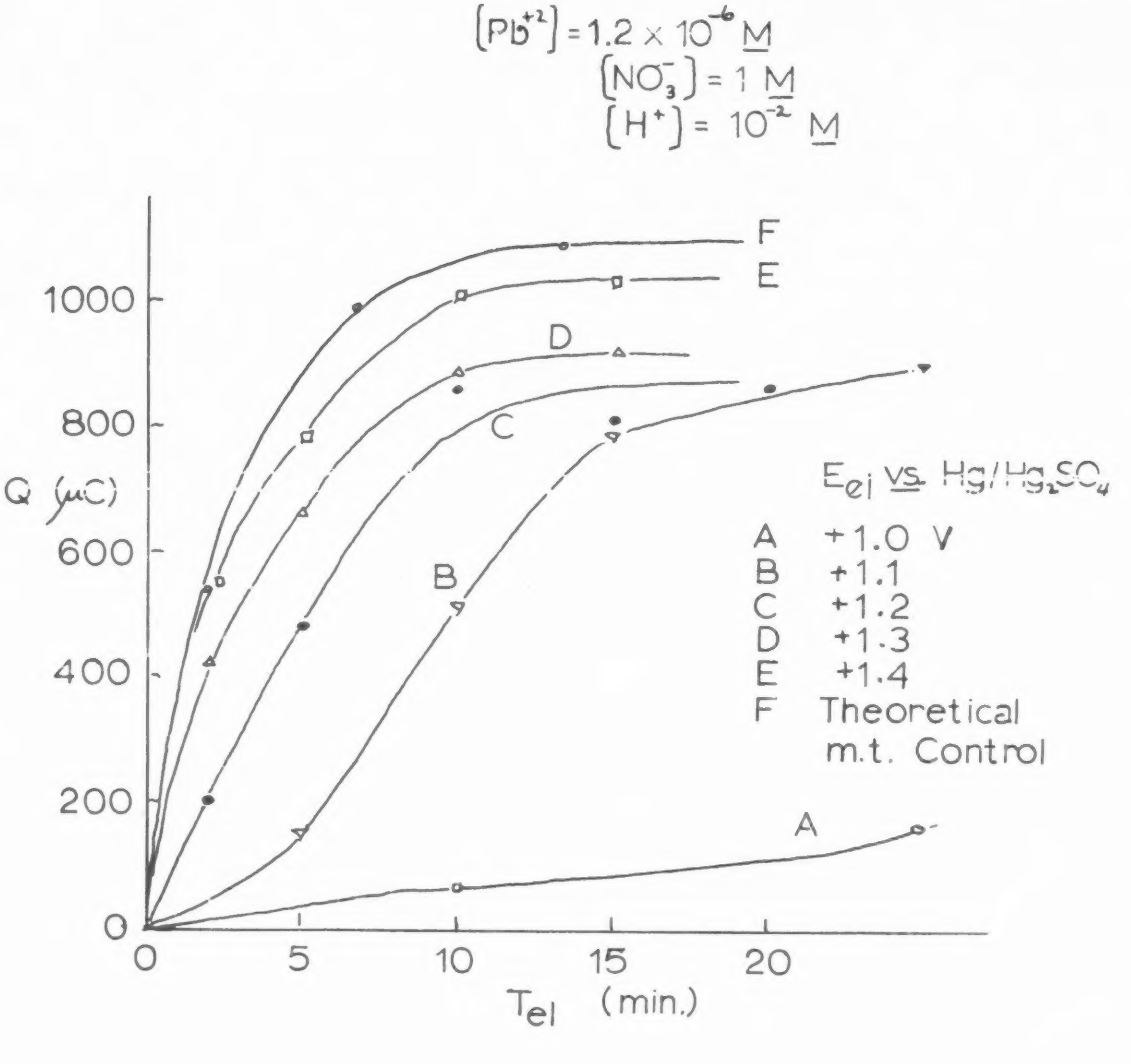


Figure 15. Lead Dioxide Deposition Rate as Function of Electrolysis Potential.

chemically strip the PbO_2:

$$PbO_2 + 2Cl^- + 4H^+ \overset{K}{\rightleftarrows} Cl_{2(aq.)} + Pb^{+2} + 2H_2O \quad E^\circ_{cell} = +0.060$$. Knowing the equilibrium constant K, one can predict the extent of chemical stripping.

Bruckenstein and Bixler (1965) derive a relationship for the rate of chemically stripping a film of silver by an oxidant in solution in terms of the equilibrium and mass transport rate. Similar equations have been derived for the PbO_2/Cl^- reaction. For example, in a solution with $[H^+] = 10^{-2}$ M, $[Pb^{+2}] = 10^{-6}$ M, and $[Cl^-] = 1$ M, it is theoretically predicted that the PbO_2 deposit will be completely stripped within 0.3 seconds if the electrode is left at open circuit. Decreasing the chloride concentration to 10^{-4} M increases the stripping time to 2800 seconds. The stripping rate is predicted to increase with decreasing pH.

Experimentally, it is possible to quantitatively deposit and strip PbO_2 from 10^{-6} M Pb^{+2} and 10^{-2} M HNO_3 in the presence of up to 10^{-4} M chloride. Higher chloride concentrations have not been investigated.

Higher chloride concentrations would also interfere with the deposition due to the anodic current produced in oxidizing the chloride to chlorine limiting the anodic polarization range.

During the pre-electrolysis, hydrogen is produced at the counter electrode. This hydrogen has been experimentally observed to chemically strip PbO_2. The cell has been designed to isolate the counter electrode in a separate compartment.

Lead dioxide is thermodynamically unstable with respect to oxidation of water: $2H_2O + 2PbO_2 + 4H^+ \rightleftarrows 2Pb^{+2} + 4H_2O + O_2 \quad E^\circ_{cell} = +0.226$; $K = 2 \times 10^{15}$. However, the rate of oxidation is kinetically slow under the conditions used. For example, after PbO_2 is deposited the electrode can be left on open circuit in 10^{-2} M HNO_3 for ten minutes without loss.

It is desirable to be able to determine lead in samples containing large amounts of phosphate. Bone samples would be an extreme example. This particular matrix causes considerable problems in the determination of lead by atomic absorption spectroscopy (AA). Cathodic stripping coulometry could complement AA for this type of sample in particular.

The presence of phosphate as high as 10^{-3} $\underline{M}$ does not interfere with the quartitative determination of 10^{-6} $\underline{M}$ Pb^{+2} in 10^{-2} $\underline{M}$ HNO_3. However, the presence of phosphate causes a change in the relative amounts stripped in the reversible and secondary peaks. With 10^{-4} and 10^{-3} $\underline{M}$ phosphate the PbO_2 deposits predominantly in the form of the monolayer directly on the tin oxide surface as indicated by the curves in Figures 16a and 16b. This is in contrast to electrolysis and stripping in the absence of phosphate shown in Figure 16c. Despite this redistribution in the PbO_2 activity, the electrolysis and stripping is still quantitative.

When larger quantities of PbO_2 are deposited in the presence of phosphate even larger amounts of PbO_2 are deposited as the monolayer. A series of stripping curves for increasing amounts of PbO_2 is shown in Figure 17. The secondary current peak in curve A is equivalent to 83% of a monolayer.

These experimental data seem to indicate that phosphate promotes the two-dimensional growth of the nuclei.

Instrumentation. To make this technique more practical and convenient a rapid method is needed to determine the stripping current integral, or charge. A semiautomatic electronic integrator has been designed, constructed and tested. The instrument integrates between two preset potential limits. After pre-electrolysis a total integral, signal plus background charge, is determined. The residual current is also integrated so that it may be subtracted from the total integral. Electronic integration agrees well with manual integration of the current-time curve with a planimeter.

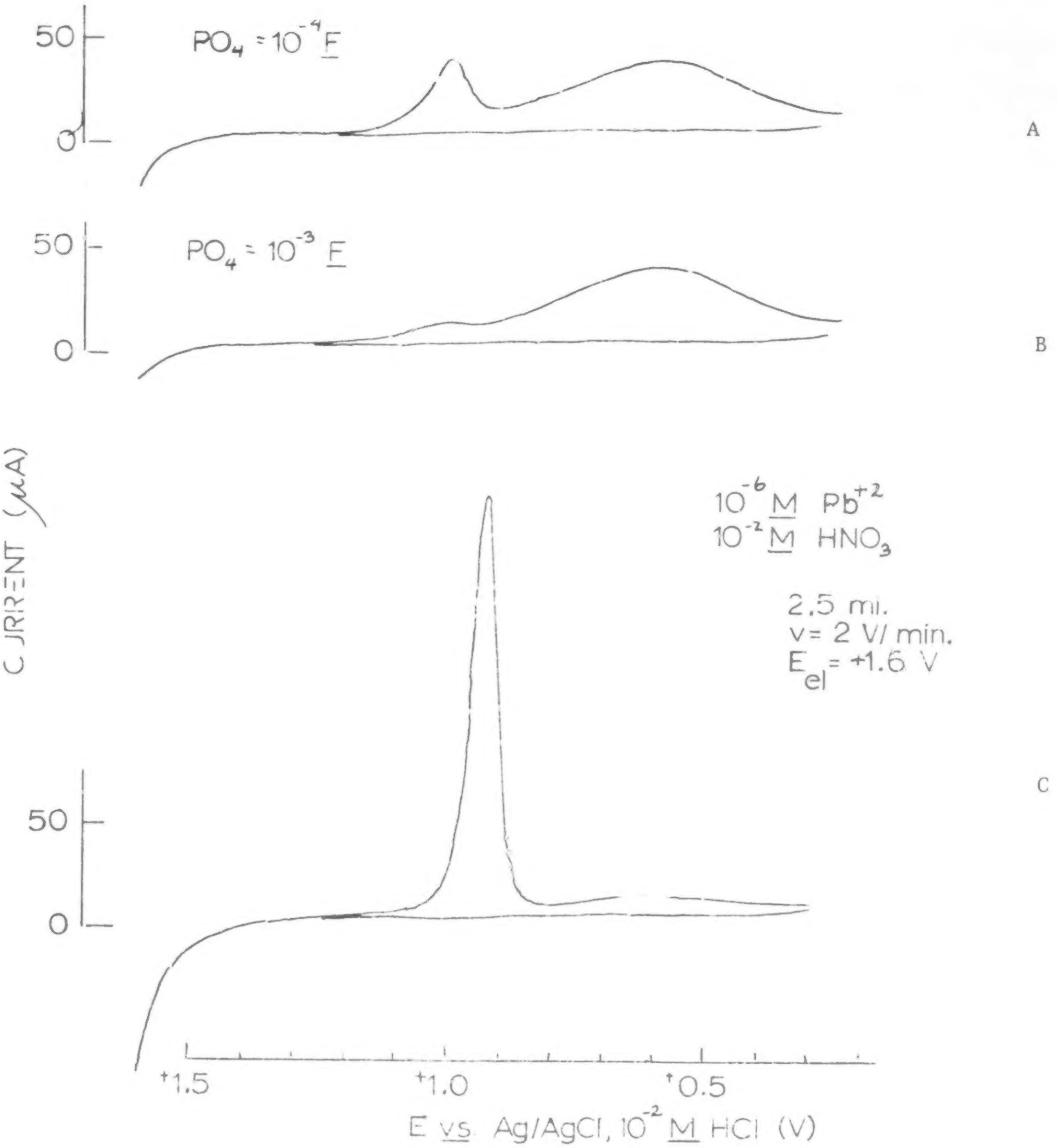


Figure 16. Effect of Phosphate on Lead Dioxide Deposition and Stripping.

a. 10^{-4} F KH_2PO_4

b. 10^{-4} F KH_2PO_4

c. Without added phosphate

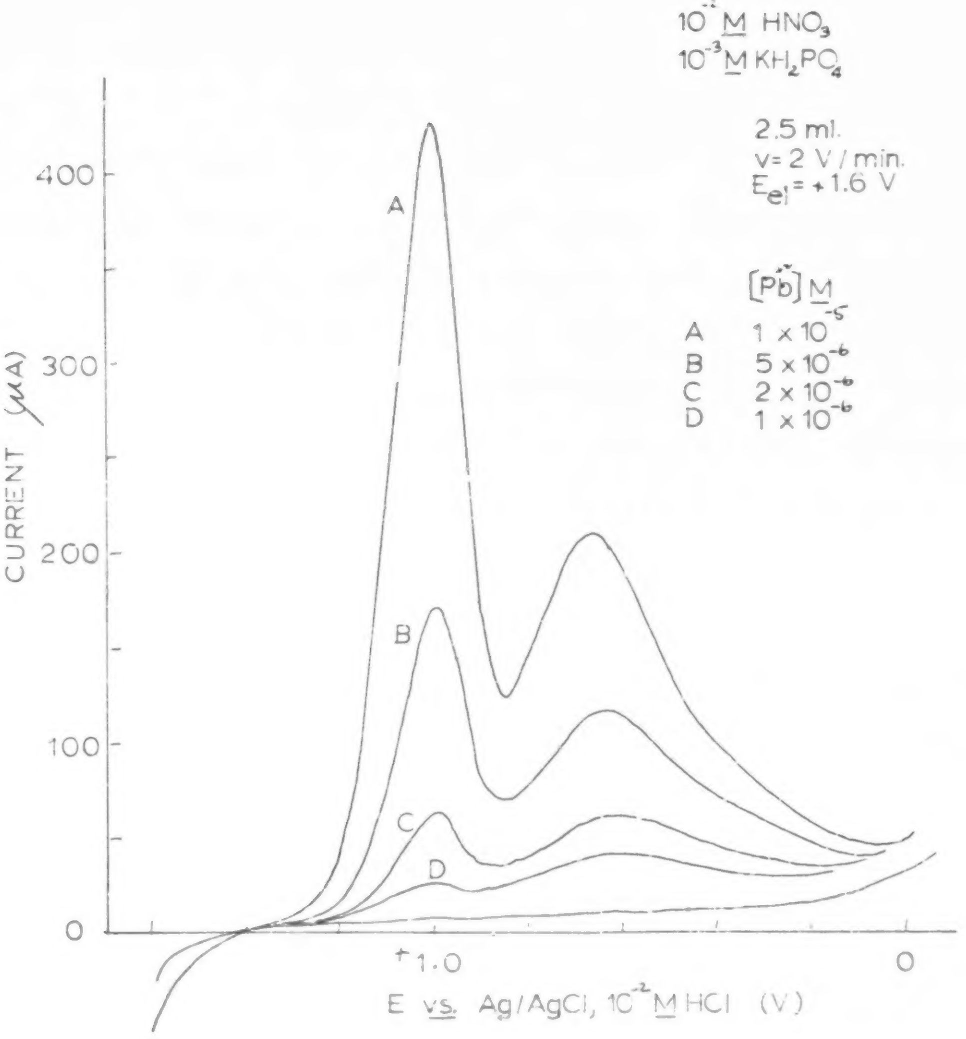


Figure 17. Effect of Phosphate at Higher Lead Concentrations

Sensitivity of Method. The sensitivity for this method using the present electrode and cell parameters is estimated to be from 5×10^{-8} to 10^{-7} $\underline{M}$ Pb^{+2}. At this level there would be 2 to 4 microcoulombs PbO_2 per square centimeter of electron area, the equivalent of 0.7 to 1.4% of a monolayer.

The sensitivity may be extended down to 5×10^{-9} to 10^{-8} $\underline{M}$ Pb^{+2} (1 to 2 ppb) by decreasing the electrode area by a factor of ten. Decreasing the electrode area would necessitate increasing the mass transport rate by a factor of ten or increasing the electrolysis time. A smaller electrode with improved mass transport properties is being designed and constructed.

d. Precision Estimates and Performance Characteristics of Electrochemical Methods.

Measures of dispersion (precision) and of central value (accuracy) are the indices by which the analytical chemist judges the worth of an analytical measurement. Such terms are also raised in every contact between the Analytical Laboratory and its users for they are of the essence in answering the perennial question: "What do these numbers mean?" The Analytical Laboratory has been asked the derived questions so frequently that it has begun a program of creating derived statistics which can readily convert to the desired answers. An additional bonus has been to reveal where the Laboratory should be placing its efforts when improving its performance. The original objective of this research was to place all the electrochemical methods on a common basis for comparison purposes and eventually to connect these with similar values for the spectroscopic methods being developed. To the present time, it has proven impossible to create a single definition of dispersion for the several electrochemical methods that do not place at least one in an untrue disadvantageous light. The basic reason for this is that each method behaves differently as the substance measured is reduced to the vanishing point. For example, atomic absorption measurements are random flame noise limited while visible-UV solution absorption spectroscopy is generally electronic shot noise limited. On the other hand, the classical noise factor in an electrochemical measurement is so low as to excessively promote the methods beyond reality if the usual signal-to-noise criterion is employed. It is more often the case that electrochemical methods become limited by a change in the form of the analog output which is not accomodated by the theory at higher concentrations. A case in point is anodic stripping voltammetry with the Hanging Mercury Drop Electrode (HMDE). At typical concentrations of 20-100 ppb lead, one obtains a curve similar to Figure 6 in which the event occurs as a peak. If the concentration of lead is decreased, it is found that the peak becomes a plateau due to the fact that the back side of the peak does not return to the baseline within the time of measurement. In this case, it is necessary to define the detection limit so that the commonly accepted measurement, peak height, will still be valid. Table 5 lists the brief definitions used for the Limits of Detection and Sensitivity for the several methods.

3. Standardization

The usefulness of analytical results is dependent on a knowledge of the precision and accuracy of reported values. The precision of analytical measurements and results can be readily determined and controlled by internal means. Measures of accuracy, on the other hand, are more difficult to obtain and require resources not usually available in a single laboratory.

Assessment of accuracy usually involves analysis by several independent methods, in the same laboratory, with the assumption that the degree of agreement between methods is a function of accuracy; or participation in an inter-laboratory comparison of analytical results obtained on carefully prepared samples--again with the assumption that if agreement is good, so must be the accuracy. The third and probably the most useful means of assessing accuracy is through the analyses of carefully prepared standard samples that have been exhaustively analyzed by numerous methods without regard to expenditure of effort, and the results have been certified by a central organization such as the National Bureau of Standards.

In the determination of trace heavy metals in environmental substances, the laboratory has only two independent methods that are applicable to a given material; and so measures of accuracy rely on the availability of standardized materials and/or participation in interlaboratory comparison studies.

In the determination of trace heavy metals in environmental substances, the laboratory relies on spectrochemical and electrochemical methods. The two methods are optimally employed at widely different concentration ranges of the analyte. Table 7 lists comparative results by atomic absorption and anodic stripping voltammetry on routine analyses at the concentration interfaces between the two methods.

a. Round-Robin Interlaboratory Comparison. Sets of twelve samples representing plant and animal tissues, soil, and air filters were distributed on a "round-robin" basis by R. K. Skogerboe of Colorado State University to seven institutions involved in the analysis of environmental materials. Duplicate determinations for lead were to be made by methods of choice, and the results reported to Dr. Skogerboe for statistical evaluation.

A meeting was held on the afternoon of March 9 during the Pittsburgh Conference between representatives of the participating institutions to

Table 7. COMPARISON OF ANALYTICAL RESULTS BY ATOMIC ABSORPTION AND ANODIC-STRIPPING VOLTAMMETRY

		Lead Concentration (ppm)		
Sample Type	Sample No.	AA	ASV	Difference (AA - ASV)
Rat Muscle	253	0.36	0.27	+0.09
	255	0.41	0.55	-0.14
	102	0.43	0.32	+0.11
	104	0.40	0.35	+0.05
Rat Blood	31	0.19	0.25	-0.06
	36	0.22	0.19	+0.03
	41	0.62	0.73	-0.11
	42	0.45	0.45	0.00
Rat Bone	108	0.44	0.16	+0.28
	111	0.50	0.93	-0.43
	113	0.36	0.32	+0.04
	114	0.52	0.41	+0.11
	115	0.36	0.70	-0.34
	116	0.25	0.29	+0.04
	117	0.28	0.10	+0.18
	291	0.25	0.31	-0.06
	295	0.23	0.30	-0.07
	297	0.29	0.49	-0.20
Rat Kidney	48	0.20	0.14	+0.06
	49	0.25	0.13	+0.12
	50	0.16	0.13	+0.03
	51	0.17	0.10	+0.07
	52	0.15	0.08	+0.07
	53	0.17	0.13	+0.04
	54	0.23	0.11	+0.12
	55	0.12	0.10	+0.02
	56	0.15	0.12	+0.03
	57	0.15	0.18	-0.03
	58	2.3	2.2	+0.10
	59	2.8	2.6	+0.20
Rat Spleen	73	0.13	0.12	+0.03
	74	0.19	0.19	0.00
Air Filter (1)	63F	16.0	16.5	-0.50
	52C	2.5	2.4	+0.10
	3A1	0.47	0.50	-0.03

(1) Concentrations expressed as micrograms per cm^2

Table 8. SUMMARY OF ANALYTICAL RESULTS OF ROUND-ROBIN INTERLABORATORY COMPARISON

	Lead Concentration (ppm)					
	Participating Laboratory(1)					
	A	B	C	D	Grand Mean	Sig. Difference from Mean (2)
Orchard leaves	41.3	42.9	42.5	42.3	42.3 *	No
Tomato leaves A	-	4.1	6.6	4.85		
Tomato leaves B	-	3.75	4.45	4.8	4.4	No
Soil A	26.6	22.5	27.0	24.4	25.1	No
Soil B	222.	220.	250.	256.	237.	No
Soil C	97.	94.6	135.	98.	106.	Borderline
Dried Blood A	0.80	1.75	1.45	1.40	1.35	No
Dried Blood B	1.08	1.60	1.40	1.55	1.40	No
Bovine Liver	-	0.93	0.29	0.77	0.66 **	(3)

NOTES:

(1)

Code Letter	Laboratory
A	Ethyl Corporation
B	University of Missouri
C	University of Illinois
D	Colorado State University

(2) By t test at the 95% C.L.

(3) Insufficient data

* N.B.S. SRM 1571 certified value: 44. ppm

** N.B.S. preliminary value: 0.37 ppm

discuss the results. Present were:

Manual Brandt	Ethyl Corporation
Cyrus Feldman	Oak Ridge National Laboratories
S. R. Koirtyohann	University of Missouri
R. K. Skogerboe	Colorado State University
R. S. Vogel	University of Illinois

A compilation and statistical evaluation of the intercomparative results was presented by Rod Skogerboe who had acted as administrator of the round-robin exchange. A condensed summary of the reported values is given in Table 8. A complete report of the data and correspondence appears in the Appendix of this section.

Statistical tests for inter- vs. intra-laboratory differences indicated no significant bias in analytical results at the 95% C.L. between the reporting laboratories for all materials except Bovine Liver. The method of statistical evaluation was based on the deviation of duplicate results from the pooled averages. Pooled analytical data indicated a relative standard deviation between laboratories ranging from a low of 3% for Orchard Leaves SRM to a high of 48% (estimated) for Bovine Liver. Results from the simulated air-filter samples had such wide dispersion that no statistical evaluation was made. Dr. Skogerboe had used a carefully-prepared suspension of lead chromate filtered in quantized volumes through membrane filters which were then dried. The large variations found between results of a seemingly straightforward analysis indicated possible problems in filter preparation or sampling. Some of the problems related to preparation of reliable synthetic filter standards were discussed.

Analytical Methods. Plant and animal tissue samples were wet ashed with HNO_3 and $HClO_4$ or H_2SO_4 mixtures by all reporting laboratories except the University of Illinois which employed dry ashing and HCl digestion. Soil samples were digested with HCl or HNO_3. A preliminary separation of lead from the sample matrix was made by Ethyl Corporation and the University of Missouri via the traditional complexation/MIBK extraction route prior to the analytical measurement. The University of Missouri employed a micro-sample technique for the analysis of the tomato leaf sample.

Conclusions and Future Plans. The concensus of opinion among the participating laboratories was that interlaboratory agreement was surprisingly good for biological materials. Established procedures applied to homogeneous materials such as NBS Orchard Leaves (SRM 1571), in which the lead concentration (44 ppm) was relatively high, the pooled relative standard deviation from the grand mean was only 3%. On the other extreme, the corresponding deviation for Bovine Liver reflected the problems associated with very low concentrations of the analyte, and a high degree of chemical interferences.

Work needs to be done toward understanding and finding more efficient means for dealing with chemical and other interferences, and to improve analytical sensitivity and precision in the analysis of types of animal tissue in which the concentration for heavy metals is in the fractional parts-per-million range. A new provisional standard reference material, Bovine Liver - SRM 1957, to be made available soon by NBS, will be of considerable help in developing and standardizing methods for animal tissue analysis. Other biological SRM's, such as blood, now in the works at NBS, will further help to fill a critical need for reference standards to provide a common basis for standardizing analytical methods for the determination of trace elements in environmental materials.

A recent announcement from the International Atomic Energy Commission of their entry into analytical quality control services and their intentions of making available standard reference materials will be another potential source of standards of which air filters, water samples, animal tissue such as bone and blood, soils, and lake sediments will be of interest to us.

The personal interaction afforded by the round-robin exchange, aside from providing an efficient means for discussion of methodology, helped to improve the feeling of common involvement between the academic, governmental, and industrial group participants. As a result of this meeting, the Ethyl Corporation Laboratory, through Mr. Brandt, distributed a set of synthetically prepared air-filter samples for evaluation and comparison of results.

We expect to expand this type of interaction to include other laboratory groups such as at Purdue involving cadmium, at Stanford concerning mercury, and the CSU investigation of molybdenum, as well as laboratories of the State of Illinois Environmental Protection Agency.

Standardization Procedures and Equipment Calibration. The Analytical Laboratory was organized as a central analytical facility to serve the Environmental Studies Program according to the concept that the analytical needs of the Program would be best served by a single group dedicated to analytical chemistry.

A part of the overall functions of the Laboratory is to assist in the development of systematic sample collection and preliminary preparation. Part of this preparation at times involves pre-drying and ashing of samples, and in some cases digestion of ashed samples in hydrochloric acid. The Laboratory operates apparatus for redistillation of hydrochloric acid water azeotrope which is supplied to participating groups to use in sample preparation.

A source of ultra-high-purity mineral acids available to the Program is the National Bureau of Standards, with whom arrangements are being made for nominal quantities at relatively low cost.

The Laboratory maintains a carefully standardized thermocouple potentiometer for use as a service in temperature calibration of ovens used at various locations for the initial dry-ashing of plant and animal materials.

An important consideration in accuracy of analytical results is the control of sample-matrix concentration in solutions submitted for analysis. Written analytical procedures are distributed to those groups in other locations on campus involved in such preparation so that uniform and known practices are employed through all steps of the analytical procedures.

C. Routine Processing Activities

1. General Laboratory Operation

With the addition of Miss Bonnie Blanding to provide secretarial and clerical assistance on a shared-time basis with Administration, the secretarial and clerical "paper work" of the Laboratory was placed on a centralized basis.

Procedures for systematic handling of analysis requests, receiving and logging of samples, preparation of analytical reports, and disposition of samples have been put into routine use. A weekly follow-up tally of analysis requests on hand provides useful feedback information on the state of affairs. Estimated schedules of analysis with respect to sample types and time distribution that will be requested during the current phase of the program have been compiled to aid the laboratory in determining manpower and equipment needs. Figure 18 is an operational flow chart of the Analytical Laboratory.

a. Analytical Reports. A dual-purpose printed form designed to serve both as the request for analysis and the analytical report has been in use since October 1971. All entries, including analytical results, are made directly by the persons involved, thereby minimizing clerical time and transcription errors.

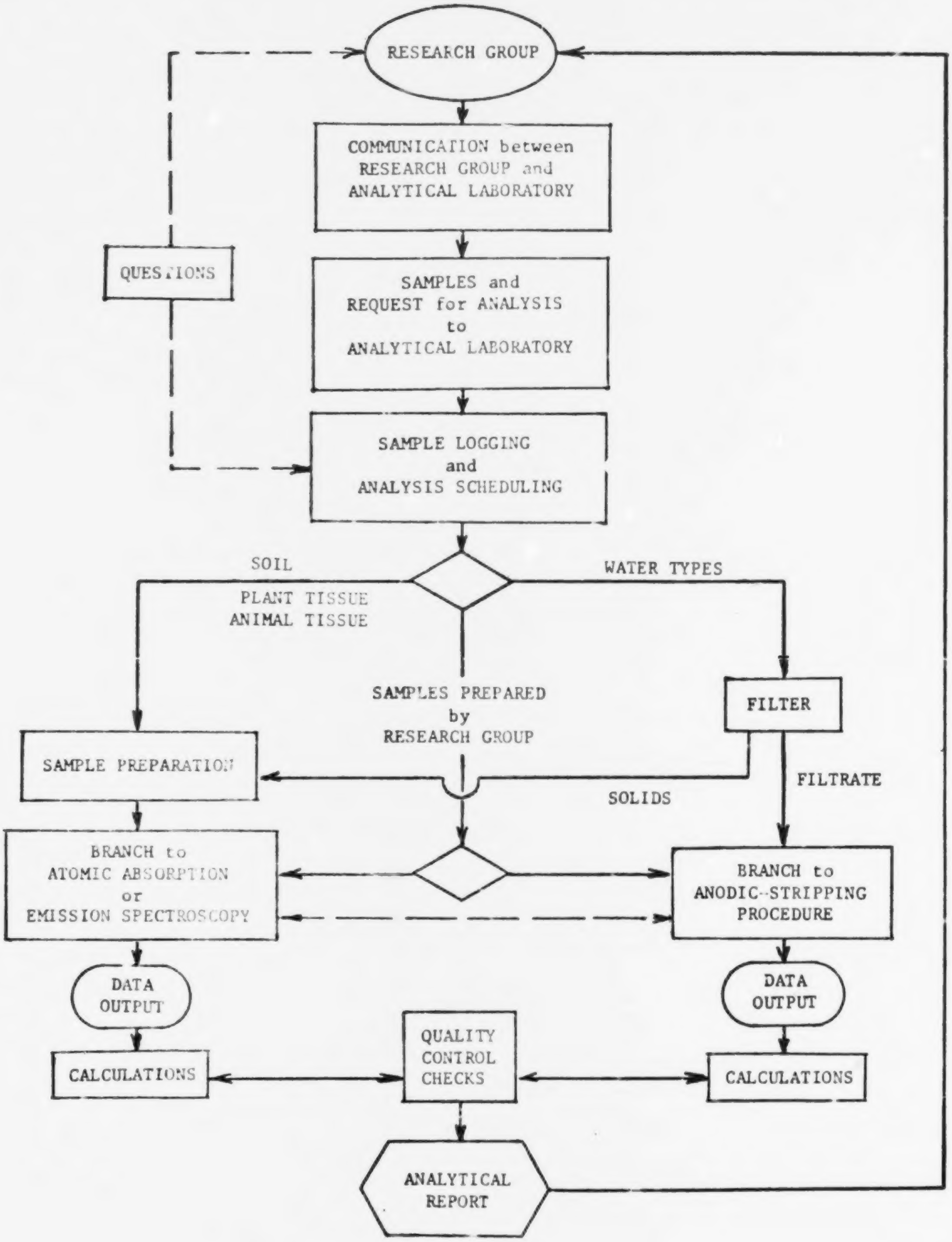

RESEARCH GROUP
COMMUNICATION between RESEARCH GROUP and ANALYTICAL LABORATORY
QUESTIONS
SAMPLES and REQUEST for ANALYSIS to ANALYTICAL LABORATORY
SAMPLE LOGGING and ANALYSIS SCHEDULING
SOIL
PLANT TISSUE
ANIMAL TISSUE
WATER TYPES
SAMPLES PREPARED by RESEARCH GROUP
FILTER
SAMPLE PREPARATION
SOLIDS
FILTRATE
BRANCH to ATOMIC ABSORPTION or EMISSION SPECTROSCOPY
BRANCH to ANODIC-STRIPPING PROCEDURE
DATA OUTPUT
DATA OUTPUT
QUALITY CONTROL CHECKS
CALCULATIONS
CALCULATIONS
ANALYTICAL REPORT

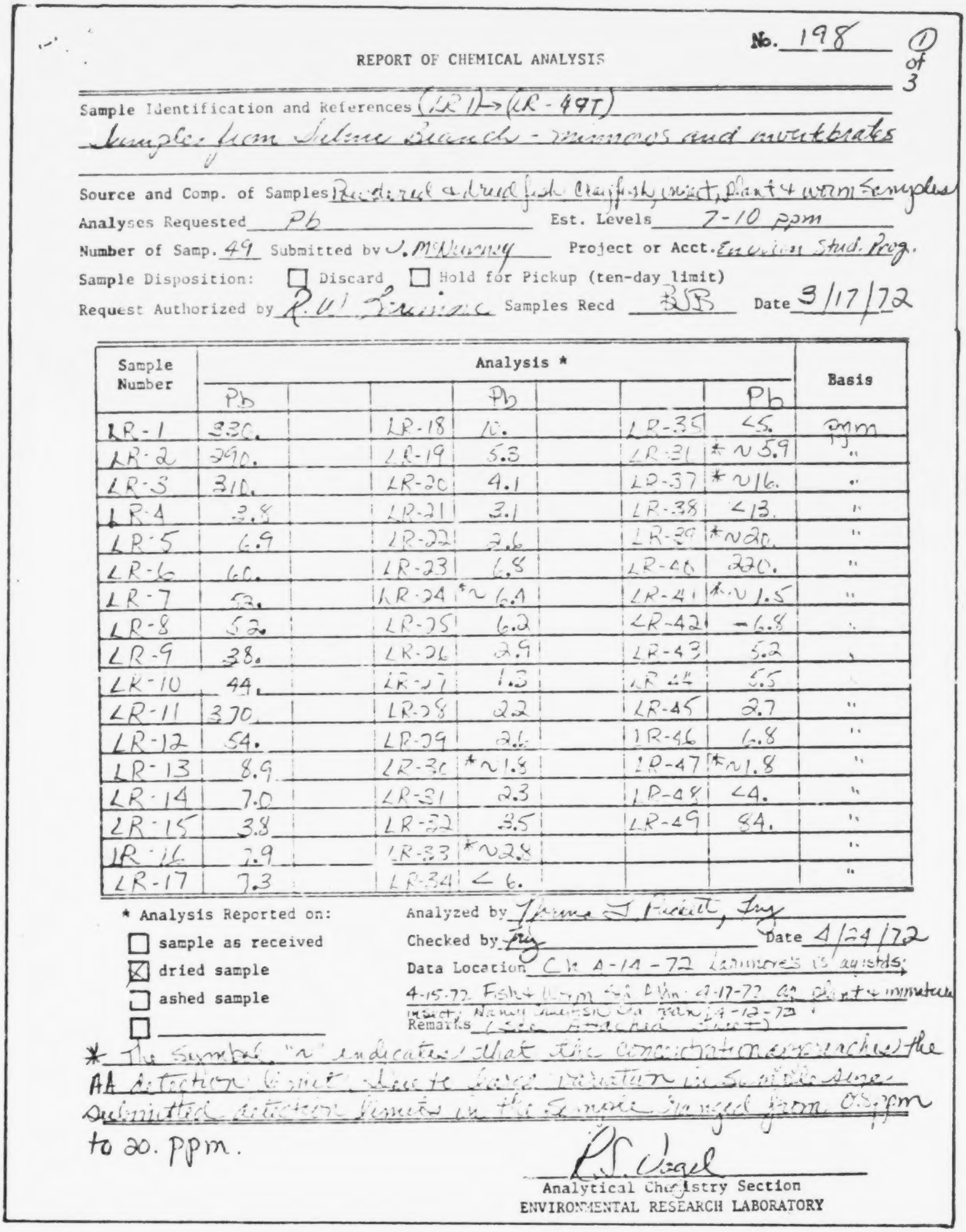
Figure 18. Operational Flow Chart of Analytical Laboratory

Typical completed forms are shown in Figures 19 and 20. The upper section of the form, filled in by the person requesting the analysis, provides the laboratory with information regarding sample type, analyses requested, estimated concentration, and instructions for sample disposition. The authorization signature also serves to keep the principal investigators informed of the total analytical activities of members of their groups. A sample-receiving clerk at the Analytical Laboratory checks the sample labels against the corresponding sample numbers, validates the information, enters the receiving date and assigns a sequential serial number from a log. Communication between the investigators and the laboratory, individually and through group meetings, establishes priorities in the analytical schedules. After analytical methods have been codified for given sample types, succeeding samples of the same type are processed in a routine manner according to agreed priorities.

Analysis values are entered in the analytical report form by the analyst/s who personally performed the analyses; calculations are counter-checked; and the report countersigned by a group leader. The Environmental Research Laboratory Girl-Friday enters the reporting date and sees to the distribution of the report.

The currently-used form has undergone two evolutionary changes, and with the next printing will be supplied as a four-copy snap-out.

b. Identification of Samples Submitted for Analysis. In the course of the initial period of operations, it became apparent that a simple, uniformly-used sample identification scheme was needed by which samples received from the twenty-five or more principal investigators in the Program could be identified with respect to the person requesting the analysis and the sample type (plant, animal, etc.) as well as the place of the sample in a given series of an experimental plan.



No. 198 ① of 3

REPORT OF CHEMICAL ANALYSIS

Sample Identification and References (LR 1) → (LR - 49T)
Samples from Silver Branch - minnows and invertebrates

Source and Comp. of Samples Powdered & dried fish, crayfish, insect, plant + worm samples
Analyses Requested Pb Est. Levels 7-10 ppm
Number of Samp. 49 Submitted by J. McNurney Project or Acct. Environ. Stud. Prog.
Sample Disposition: ☐ Discard ☐ Hold for Pickup (ten-day limit)
Request Authorized by R. W. Lorimore Samples Recd BJB Date 3/17/72

Sample Number	Analysis *							Basis
	Pb			Pb			Pb	
LR-1	330.		LR-18	10.		LR-35	<5.	ppm
LR-2	290.		LR-19	5.3		LR-36	*~5.9	"
LR-3	310.		LR-20	4.1		LR-37	*~16.	"
LR-4	3.8		LR-21	3.1		LR-38	<13.	"
LR-5	6.9		LR-22	2.6		LR-39	*~20.	"
LR-6	60.		LR-23	6.8		LR-40	220.	"
LR-7	53.		LR-24	*~6.4		LR-41	*~1.5	"
LR-8	52.		LR-25	6.2		LR-42	~6.8	"
LR-9	38.		LR-26	2.9		LR-43	5.2	"
LR-10	44.		LR-27	1.3		LR-44	5.5	"
LR-11	370.		LR-28	2.2		LR-45	2.7	"
LR-12	54.		LR-29	2.6		LR-46	6.8	"
LR-13	8.9		LR-30	*~1.8		LR-47	*~1.8	"
LR-14	7.0		LR-31	2.3		LR-48	<4.	"
LR-15	3.8		LR-32	3.5		LR-49	84.	"
LR-16	2.9		LR-33	*~2.8				"
LR-17	7.3		LR-34	<6.				"

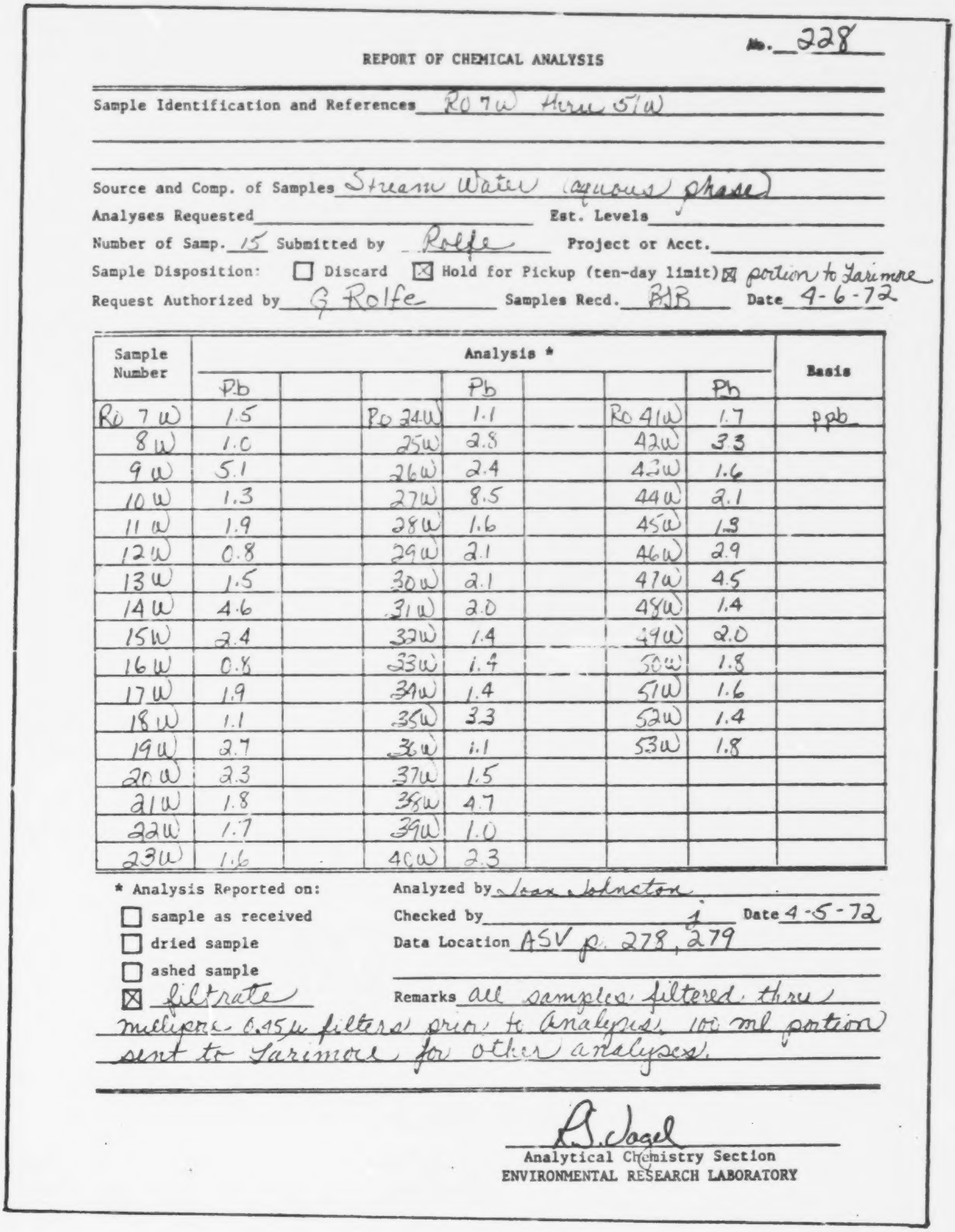
* Analysis Reported on:
☐ sample as received
☒ dried sample
☐ ashed sample
☐ ____________

Analyzed by Thomas J. Pickett, Jr.
Checked by Jr. Date 4/24/72
Data Location Ch 4-14-72 Larrimore's 15 aq stds; 4-15-72 Fish + Worm [illegible] 4-17-72 [illegible] plant + immature insect; Nancy [illegible] [illegible] 4-12-72
Remarks (See attached sheet)

* The symbol "~" indicates that the concentration approached the AA detection limit. Due to large variation in sample sizes submitted detection limits in the sample ranged from 0.8 ppm to 20. ppm.

R. S. Vogel
Analytical Chemistry Section
ENVIRONMENTAL RESEARCH LABORATORY

Figure 19. Analytical Request/Report on Dual-Purpose Form



No. 228

REPORT OF CHEMICAL ANALYSIS

Sample Identification and References RO 7W thru 51W

Source and Comp. of Samples Stream Water (aqueous phase)

Analyses Requested ______ Est. Levels ______

Number of Samp. 15 Submitted by Rolfe Project or Acct. ______

Sample Disposition: ☐ Discard ☒ Hold for Pickup (ten-day limit) ☒ portion to Larimore

Request Authorized by G Rolfe Samples Recd. BJB Date 4-6-72

Sample Number	Analysis *							Basis
	Pb			Pb			Pb	
Ro 7W	1.5		Ro 24W	1.1		Ro 41W	1.7	ppb
8W	1.0		25W	2.8		42W	3.3	
9W	5.1		26W	2.4		43W	1.6	
10W	1.3		27W	8.5		44W	2.1	
11W	1.9		28W	1.6		45W	1.3	
12W	0.8		29W	2.1		46W	2.9	
13W	1.5		30W	2.1		47W	4.5	
14W	4.6		31W	2.0		48W	1.4	
15W	2.4		32W	1.4		49W	2.0	
16W	0.8		33W	1.4		50W	1.8	
17W	1.9		34W	1.4		51W	1.6	
18W	1.1		35W	3.3		52W	1.4	
19W	2.7		36W	1.1		53W	1.8	
20W	2.3		37W	1.5				
21W	1.8		38W	4.7				
22W	1.7		39W	1.0				
23W	1.6		40W	2.3				

* Analysis Reported on:

☐ sample as received

☐ dried sample

☐ ashed sample

☒ filtrate

Analyzed by Joan Johnston

Checked by j Date 4-5-72

Data Location ASV p. 278, 279

Remarks all samples filtered thru millipore 0.45μ filters prior to analysis. 100 ml portion sent to Larimore for other analyses.

B. Vogel
Analytical Chemistry Section
ENVIRONMENTAL RESEARCH LABORATORY

Figure 20. Analytical Request/Report on Dual-Purpose Form

A sample-labeling scheme was developed to serve these needs, as well as to be compatible with eventual computer use. The labeling scheme involves an alpha-numeric code consisting of two prefix letters denoting the senior staff member authorizing the analysis request, followed by a sequential number to identify a given sample within a series and one or two suffix letters when necessary to denote a given series or the end of a series. A color-coded label categorizes the sample as to plant, animal, soil, water, or other origin according to the following code:

Sample Classification	Label Color
Animal tissue	red
Animal processes	rose
Plant tissue	green
Plant processes	chartreuse
Soil	orange
Soil fractions	yellow
Natural water	blue
Water fractions	aqua
Other	tan

c. Service Analyses Reported. During the period between May 1, 1971, and May 1, 1972, a total of 6032 analytical results were reported on samples distributed according to the categories listed in Table 9.

Analytical determinations ideally are made by atomic absorption for lead concentrations in the analytical solution of 0.3 ppm or more, and by anodic stripping at lead concentrations below that value. The concentrational cross-over point varies slightly according to sample matrix and available sample size. Since the procedure for preliminary preparation of the sample is compatible to both atomic absorption and anodic stripping methods, sample preparation time is not lost should the lead concentration in a sample fall outside of the anticipated range.

Table 9. - Analyses Performed between May 1, 1971 and May 1, 1972

Sample Classification	Analyses *
Animal	694
Plant	2035
Soil	1195
Water	1785
Other	323
Total Analyses	6032

* including multi-element analyses on a given sample.

Anodic stripping voltammetric methods provided the sole means for the determination of heavy metals at the parts-per-billion level in natural waters and in other materials where limited sample quantities resulted in fractional parts-per-million lead concentrations in the prepared sample solution.

Atomic absorption and flame emission spectrometry was applied to the analysis of soils, plant and animal tissues, suspended solids in natural waters and sewage, in membrane transfer studies, in air filters and other miscellaneous materials.

d. Water Analyses. Other work in progress includes a study of of particle size distribution of suspended matter in stream waters with respect to lead transport by suspended matter. On the basis of limited data, approximately 90% of the total lead transported by suspended matter is transported by particles greater than 5 micrometers mean diameter. Analytical results of the distribution of total lead between the suspended and the aqueous phase on approximately one hundred samples of stream water from urban and rural environments are presented in the Total Ecosystem section of this report.

The analytical separation of suspended matter from the aqueous phase is made by vaccum filtration of a 250-ml water sample through 47-mm diameter Millipore filters of 5 micrometer pore size. Lead in the aqueous phase is determined directly by anodic stripping voltammetry, and in the suspended matter by leaching the Millipore filter containing suspended matter in 10 ml of 3 M HCl and applying atomic absorption spectrometry.

D. Appendix

Correspondence and Statistical Evaluation Data on the Round-Robin Interlaboratory Comparison

ETHYL CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT
RESEARCH LABORATORIES · 1600 WEST EIGHT MILE ROAD
FERNDALE, MICHIGAN 48220

February 15, 1972

Dr. R. K. Skogerboe
Department of Chemistry
Colorado State University
Fort Collins, Colorado 80521

Dear Rod:

Listed below are results obtained on the nine samples recently sent to Gary Ter Haar and the NBS Orchard Leaves. As agreed in our February 1 telephone conversation, results on the filters are given for the total segment rather than on an area basis.

Filters	Pb, µg		
8-1-F	401		
8-2-F	237		
9-1-F	223		
9-3-F	153		
Soils	**Pb, µg/g**		**Sample wt., g**
A-5	22.3	30.9	1
B-8	230	213	1
C-2	77.9	116	1
Blood, Dried			
1A	0.81	0.78	2
11B	1.12	1.03	2
NBS Orchard Leaves	40.6	42.1	1

Attached are brief descriptions of the procedures which were used.

You had indicated that a meeting was planned during the Pittsburgh Conference to discuss results by the various laboratories. I am planning to attend the meeting on March 6, 7 and 8 and prefer March 8 for the meeting.

M. Brandt

MB/jkh
Attachments
cc: J. B. Hinkamp
G. Ter Haar

Soils

Add 25 ml of 10% nitric acid solution to the sample in a 100-ml beaker and heat to boiling. Cool and filter into a 125-ml separatory funnel and determine the lead in the filtrate using the sodium diethyldithiocarbamate (NaDDC) procedure with 10 ml of MIBK.

Filters

Transfer the filter to a 100-ml beaker. Rinse the container twice with 2-3 ml of 1% nitric acid solution and add the rinsings to the beaker. Add 5-ml of 3:2 nitric:perchloric acid solution to the beaker and oxidize the filter. Dilute to 15-20 ml and transfer the solution to a 125-ml separatory funnel (Total volume: 40-50 ml) and determine the lead using the NaDDC procedure.

Leaves and Blood

Weigh the sample in a 100-ml beaker and wet the sample with 5-10 ml of water. Add 5 ml of 5:7 nitric: sulfuric acid mixture. Heat cautiously on a hot plate until the sample chars. Transfer the beaker to a muffle furnace at 525±25° and heat 5-6 hrs. Cool. Add 2 ml hydrochloric acid and 0.5 ml of nitric acid and warm to dissolve the salts. Dilute to 20-25 ml, transfer to a 125-ml separatory funnel and determine the lead by the NaDDC procedure (10 ml MIBK for leaves; 5 ml MIBK for blood).

DETERMINATION OF LEAD BY ATOMIC ABSORPTION

Apparatus

1. Separatory funnel, 125-ml.
2. Small bottle (1/2-ounce plastic preferred).
3. Atomic absorption spectrophotometer.
4. pH meter.

Reagents

1. Ammonium hydroxide.
2. Citric acid, 50%.
3. Methyl isobutyl ketone.
4. Sodium diethyldithiocarbamate (NaDDC) 1%, filtered.

4a. Ammonium pyrollidinedithiocarbamate (APDC) 2%, filtered, prepared fresh daily.

5. Thymol Blue Indicator. Dissolve 0.219 g of water soluble indicator in 500 ml of water.
6. Potassium cyanide, 10%.
7. Lead standards.
   - a. 1000 ppm. Dissolve 1.5985 g of $Pb(NO_3)_2$ in 0.8% nitric acid (V/V) solution and dilute to 1000 ml with 0.8% nitric acid solution.
   - b. 50 ppm. Dilute 5 ml of 1000 ppm solution to 100 ml with 0.8% nitric acid solution.
   - c. 5 ppm. Dilute 10 ml of 50 ppm solution to 100 ml with 0.8% nitric acid solution.

Sodium diethyldithiocarbamate (NaDDC) Procedure

1. Add 2-10 drops of 50% citric acid solution.	1. The sample solution of this point will be in a separatory funnel from the final step of a sample preparation procedure. Sufficient citric acid is used to keep the salts in solution.

2. Add 5 drops of thymol blue solution and concentrated ammonium hydroxide to the blue color of the indicator.

3. Add 5 ml of 10% potassium·cyanide solution.

4. Add 2 ml of filtered sodium diethyldithiocarbamate, 5.0 or 10.0 ml of methylisobutyl ketone (MIBK) and shake for one minute.

Alternatively, APDC may be used as chelating agent. This is particularly attractive for analyzing materials high in alkaline and/or alkaline ~~with~~ earth salts. See APDC procedure.

5. Allow the phases to separate, discard the aqueous phase and transfer the MIBK solution to a small plastic bottle of convenient size. Cap the bottle and analyze within eight hours using the instrument manufacturer's recommended instrumental operating conditions.

6. Prepare a calibration curve by adding 0 to 50 µg of lead to separatory funnels and following the above procedure (Steps 1 through 5).

Analyses should be run in the linear portions of the curve. If necessary, adjust the sample sizes.

Ammonium Pyrollidine Dithiocarbamate (APDC) Procedure

1. Adjust the pH to 3 to 3.5 with ammonium hydroxide using a pH meter.

2. Add 1 ml of APDC solution.

3. Add 5.0 or 10.0 ml of MIBK, shake for 1 min, allow the phases to separate and discard the aqueous phase.

4. Transfer the MIBK solution to a plastic bottle of suitable size and analyze within eight hours.

Calibration and blank determinations should be made comparable to that given for the NaDDC method.

Calculate the lead content of the sample as follows:

$$\text{Pb}, \mu g/g = \frac{ABC}{DW}$$

where:

A = µg of Pb per ml of MIBK (Standard)

B = Volume of MIBK for the sample, ml

C = Chart units for the sample

D = Chart units for the standard.

W = Weight of sample, g.

February 21, 1972

Dr. R. K. Skogerboe
Dept. of Chemistry
Colorado State University
Ft. Collins, Colorado

Dear Rod:

The following results were obtained from the reference samples which you prepared for circulation to NSF grantees. We did not deviate from the instructions except that the bovine liver was not dried. The moisture content is reportedly only about 1.5% and, judging from the spread between duplicates, is unlikely to be significant.

Sample	Pb 1st run	2nd run	Method
orchard leaves	44.2 µg/g	41.7 µg/g	FAA
A0-1	22.1 µg/g	22.8 µg/g	FAA
B10-1	221 µg/g	219 µg/g	DA FAA
C10-1	95.0 µg/g	94.2 µg/g	FAA
9-2-F	12.4 $\mu g/cm^2$		FAA
9-4 F	8.9 $\mu g/cm^2$		FAA
Bovine Liver	1.07 µg/g	.79 µg/g	EXT.
Tomato Leaves	4.1 µg/g	4.1 µg/g	Boat
" "	3.8 µg/g	3.7 µg/g	Boat
29 B	1.7 µg/g	1.5 µg/g	Ext.
7 A	1.7µg/g	1.8 µg/g	Ext.

FAA Flame atomic absorption
EXT. APCD-MIBK Extraction, FAA determination
Boat Standard additions using P.E. Sampling boat.

These are the same figures that I gave you by phone February 18, 1972. I look forward to comparing all results in Cleveland.

Sincerely,

S. R. Koirtyohann
Associate Professor

cc: J. O. Pierce
Tom Clevenger

Summary of Intercomparison Results

The results presented below are referenced to laboratory numbers which are as follows.

1. Ethyl Corporation
2. University of Missouri
3. University of Illinois
4. Colorado State University
5. U.S.G.S - Denver.

A. Blood Samples designated by arabic numeral A or B.

Laboratory No.	A (mean)	B (mean)
1	0.81, 0.78 (0.80) ug/gm	1.12, 1.03 (1.08) ug/gm
2	1.70, 1.80 (1.7$_5$)	1.70, 1.50 (1.60)
3.	1.10, 1.80 (1.4$_5$)	1.40, 1.40 (1.40)
4.*	1.40, 1.40 (1.40) 1.70, 1.20 (1.4$_5$)	1.50, 1.60 (1.5$_5$) 1.70, 1.30 (1.50)
Grand Mean	1.37	1.43

* Samples submitted to the lab under different numerical designations.

An estimate of the measurement precision was obtained by computing the standard deviation of duplicate results from the laboratory averages (pooled data). An estimate of the total error was determined by calculating the deviations of each value from the grand means. The results are as follows.

Source of Error	Sample A	Sample B
1. Within Lab (measurement)		
Standard Deviation, s_m	0.20	0.11
Relative Std. Dev., C_m	14.9%	7.7%
2. Between Lab (overall)		
Std. Deviation, s_T	0.39_5	0.22_6
Rel. Std. Dev, C_T	28.9%	15.8%
3. Statistical Comparison		
Calculated Ⓕ Statistic	3.75	4.20
Tabular Ⓕ Statistic	6.0	6.0
Significant Difference at 95% Level?	no	no

Note: Samples A and B are pools from 10 individuals each and should result in analyses that agree quite well.

B. Bovine Liver. Only 3 labs provided results.

Laboratory No.		µg Pb/gm (mean)
U of Mo	2.	1.07, 0.79 (0.93)
U of I	3	0.34, 0.24 (0.29)
CSU	4	0.78, 0.76 (0.77)
Grand mean.		0.66
s_m, C_m		0.095, 14.3%
s_T, C_T		0.31_8, 48.2%

The NBS results are needed to establish significance here.

C. N.B.S Leaf Standards.

		µg Pb/gm (mean)	
	Laboratory Number	Orchard	Tomato.
Ethyl	1	40.6, 42.1 (41.3)	—
U of Mo	2.	44.2, 41.7 (42.9)	a) 4.1, 4.1 (4.1) b) 3.8, 3.7 (3.7_5)
U of I	3.*	44, 41 ($42._5$)	a) 5.9, 7.3 (6.6)** b) 4.0, 4.9 (4.45)
CSU	4.	41.9, 42.8 (42.3)	a) 5.0, 4.7 (4.8_5) b) 4.7, 4.9 (4.8)
Grand Mean		42.3	4.4***
s_m, c_m		1.14, 2.7%	0.36, 8.3%
s_T, c_T		1.30, 3.1%	0.51, 11.6%.

* Values reported were on an as received basis. We corrected them for a 4.4% water content as determined at CSU. Comparison with water content obtained at other labs is necessary to validate this correction.

** These values were rejected as an outlier set.

*** Computed assuming a) and b) were the same sample...

The between lab error is not significantly greater than the within lab error at the 95% level.

D. Soils.

Laboratory No.	ug Pb/gm (mean) A	B	C
1	22.3, 30.9(26.6)	230, 213(222)	77.9, 116(97)
2	22.1, 22.8(22.5)	221, 219(220)	95, 94.2(94.6)
3	28, 26 (27)	260, 240(250)	lim. 140, 130(135)
4*	24.2, 24.6(24.4) 23.9, 25.9(24.9)	262, 251(256) 263, 264(264)	95.9, 100(98) 100, 102(101)
USGS 5. a.**	56	300	140
b.***	30	200	150
Grand Mean	25.1	242	105
s_m, C_m	2.15, 8.6%	6.74, 2.8%	9.35, 8.9%
s_T, C_T	2.75, 11%	20.2, 8.4%	18.3, 17.4%
$s_T^2 > s_m^2$ at 95% level?	No.	No.	borderline.

* Samples submitted to lab under different numerical designations as a blind check

** Extraction, AAS method, values rejected

*** Step-estimation Emission-Spec. method, values rejected

E. Filters.

Preparation of a real filter sample of sufficient homogeneity by grinding was found to be nearly impossible. The filters sent out were synthetically prepared by a method reported to be highly accurate and reproducible. Our preliminary check on this before sending out samples suggested that this was true. The results received, however, and later checks dispute this. Conclusion: The method of filter preparation is either not reproducible or few of us have acceptable methods for doing lead on filters. Our study suggests the former.

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

February 21, 1972

Professor R. K. Skogerboe
Department of Chemistry
Colorado State University
Ft. Collins, Colorado 80521

Dear Rod,

Enclosed are eight copies of our analytical data and results on the twelve round-robin samples distributed by yourself and NBS.

With the exception of the three air filter samples, analyses were performed on duplicate portions of the samples. The estimated lead concentration in the Bovine Liver sample would have indicated a larger sample size than was available.

We generally prefer to work at a concentration of at least 1 ppm solution concentration. With the exception of the liver sample, the samples provided permitted us to follow our general procedures.

With regard to a meeting time, I would prefer a time during Wednesday afternoon or Thursday morning. I am looking forward to a useful (and hopefully open) discussion of experiences and results among the participants. It certainly is a worthwhile project, and I hope round-robins will be a continuing thing.

Yours very truly,

Ray

Raymond S. Vogel

RSV/bjb

cc: Dr. A. M. Hartley
Dr. W. von Fischer
Dr. H. A. Laitinen

Atomic-Absorption Determination of Lead in Round-Robin Plant and Animal Tissue, Soil and Air-Filter Samples

1. Summary of Method

Tissue and soil sample portions were dry ashed, digested in 3N redistilled HCl, and the solution phase analyzed by atomic-absorption spectroscopy. Corrections were made for non-specific absorption.

Air-filter samples were digested in a mixture of 10 ml HNO_3 + 1 ml $HClO_4$ + 10 ml H_2O and taken just to dryness. The residue was dissolved in 20.0 g deionized water. A reagent blank was carried through the procedure. Lead determinations were made by both atomic absorption and anodic stripping voltammetry.

2. Apparatus

Beckman 440 single-beam AA spectrophotometer, 10-cm slot laminar flow burner (heated chamber).

Muffle furnace w/calibrated temperature controller

100 ml tall-form lipless Pyrex beakers

3. Reagents

Hydrochloric Acid - 6N redistilled in Pyrex

Deionized Water - through Barnstead Standard D 0803 cartridge

4. Standards

1000 ppm Pb (w/w) Stock Solution was prepared by dissolving 1.5985 g $Pb(NO_3)_2$ in deionized water to total 1000 g. Working standards were prepared according to the following schedule:

Pb (ppm)	Parts Std. Sol'n.	+	Parts H_2O
100	1 X 1000 ppm		9
50	1 X 100 ppm		1
20	1 X 100 ppm		4
10	1 X 100 ppm		9
5	1 X 10 ppm		1
2	1 X 10 ppm		4
1	1 X 10 ppm		9
0.5	1 X 1 ppm		1
0.2	1 X 1 ppm		4

5. Condensed Procedures

Sample portions (as received) were weighed into tared 100-ml tall-form lipless Pyrex beakers and placed in a muffle furnace at room temperature. The temperature was raised to 490°C in a programmed four-hour period, and held at that temperature for five hours. Bovine liver samples were ashed at 450°C.

The ash weight was recorded, a measured portion of 3N HCl was added, the beaker was covered with a Pyrex watch glass and placed on a hot plate to digest at approximately 85°C for three hours.

The digested sample was diluted with deionized water to a predetermined weight, and the resultant solution was analyzed by the conventional atomic-absorption standard addition method of two incremental additions. A correction was applied for non-specific absorption.

A summary of the analytical parameters and instrumental conditions is given in Section 6 and in Table 1.

6. Instrumental Conditions

Wavelength (Pb)	283.3 nm
Wavelength (non-spec. abs.)	287.7 nm
Spectral Bandwidth	0.5 nm
Lamp Current	15 mA
Burner	Beckman laminar flow w/heater ON
Fuel-Oxidant	C_2H_2-Air
Burner Height	11 mm below optical axis
Flame Cell	10 cm; triple pass
Aspiration Rate	4.4 g/min (pump injection)

Anodic Stripping Voltammetric Determination of Lead in Round-Robin Plant and Animal Tissue, Soil and Air-Filter Samples

1. Procedure

The sample solutions prepared for atomic abosorption measurements were further diluted with deionized water (see schedule in Table 1), and anodic stripping voltammetric measurements were made of a series of standard additions of 10 ppb Pb increments to 10-ml aliquots of the previously diluted sample solutions. Measurements were performed under instrumental conditions described below. A 1-minute potential hold step at -300 mV at the end of each measurement was used to completely strip residual Pb from the electrode between repetitions. The plating-stripping cycle was repeated three times for each analytical determination.

Lead concentrations in the original solutions and samples were calculated on the basis of the incremented current changes upon standard additions.

2. Instrumentation

Environmental Research Associates MASA Model 2014.

3. Instrumental Conditions

Sample Size	10 ml
Plating Electrode	mercury-coated graphite electrode
Plating Potential	-900 mV vs. Ag/AgCl, 0.2M KCl
Stripping Sweep Rate	40 mV/sec.

Table 1. Summary of Analytical Parameters and Results on 2-15-72 Round-Robin Samples(1)

				Lead Concentrations		
Sample	Sample Size (as rec'd)	Ash Wt. (g)	Sol'n. Wt. (g)	Sol'n. Conc.(2) (ppm)	Non-Specif. Abs. (ppm equiv.)	Sample Basis(4) (as rec'd)
Orchard Leaves (SRM 1571)	2.00	0.188	40.0	2.2	0.18	44. ppm
	2.00	0.188	40.0	2.0	0.19	41. ppm
Tomato Leaves (A)	6.01	1.161	12.0	3.1	2.0	6.2 ppm
	6.00	1.153	12.0	3.8	2.0	7.6 ppm
Tomato Leaves (B)	6.01	1.151	12.0	2.1	1.7	4.2 ppm
	6.02	1.160	12.0	2.5	1.9	5.1 ppm
Soil Sample A-6	4.00	3.843	40.0	2.8	0.62	28. ppm
	4.00	3.846	40.0	2.5	0.55	26. ppm
Soil Sample B-3	4.00	3.822	40.0	26.	0.70	260. ppm
	4.00	3.826	40.0	24.	0.70	240. ppm
Soil Sample C-9	4.00	3.817	40.0	14.	0.80	140. ppm
	4.00	3.818	40.0	13.	0.80	130. ppm
NBS Bovine Liver	7.00	0.773	10.0	0.24	0.65	0.48 ppm
	7.00	0.755	10.0	0.17	0.65	0.24 ppm
Dried Blood 6-A	8.00	0.601	16.0	0.55	0.30	1.1 ppm
	8.00	0.655	16.0	0.92	0.32	1.8 ppm
Dried Blood 16-B	8.01	0.727	16.0	0.72	0.29	1.4 ppm
	8.00	0.641	16.0	0.72	0.32	1.4 ppm
Air Filter 3-A-1	15.9 cm^2		20.0	0.50 (AA)		0.63 $\mu g/cm^2$ (AA)
			(Sol'n. diluted 1 + 9)	0.047 (ASV)		0.60 $\mu g/cm^2$ (ASV)
Air Filter 5-2-C	13.8 cm^2		20.0	2.7 (AA)		3.9 $\mu g/cm^2$ (AA)
			(Sol'n. diluted 1 + 99)	0.024 (ASV)		3.4 $\mu g/cm^2$ (ASV)
Air Filter 6-3-F	11.5 cm^2		20.0	16.3 (AA)		28. $\mu g/cm^2$ (AA)
			(Sol'n. diluted 1 + 99)	0.165 (ASV)		29. $\mu g/cm^2$ (ASV)

Notes:

(1) Except for Air Filter samples, duplicate sample portions were analyzed.
(2) Concentrations in solution on which the analytical determination was made. All Sol'n. Conc. are corrected for non-specific absorption.
(3) ASV values compensated for a reagent blank of 0.48 µg Pb.
(4) Results are reported on the samples as received, not on the dried sample. Compensation for standard moisture value is to be applied.

E. Personnel

Capabilities Expansion

Dr. A. M. Hartley
R. S. Vogel

Methods Development

Dr. A. M. Hartley
R. S. Vogel
Loretta Chessmore
Robert Fry
Joan Johnston
Norma Puckett

Noel Watkins)
Dr. H. A. Laitinen) Cathodic Stripping

Routine Processing Activities

R. S. Vogel

REFERENCES

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IV. TOTAL ECOSYSTEM

INTRODUCTION

The goal of the total ecosystem group is to determine the input, output, and residency states of lead in the watershed ecosystem. These data will serve as the basis for a lead transport model currently being developed by the modeling team.

The total ecosystem also serves as an overall framework within which basic studies of effects or fluxes can be accomplished. Laboratory studies such as those of the plant processes group or the continuum group are detailed experiments carried out within the total ecosystem framework at a lower organizational level. These types of studies provide necessary information for understanding the effects and flow of lead in the total ecosystem.

TOTAL ECOSYSTEM STUDIES

A. Input Measures

The primary source of lead entering the watershed is from the consumption of gasoline containing tetraethyl lead. The total amount of lead introduced into the watershed through the combustion of leaded fuel is a function of vehicular miles of travel within the watershed, average lead content of the fuel, and amount of fuel consumed per vehicle mile of travel. Road sampling has been done by the traffic monitoring team to determine the volume of traffic in the study area. Volume information then has been converted to an estimate of vehicle miles of travel, which in turn provided an estimate of the motor fuel consumption in the watershed.

An additional lead input to the rural component is from leaded fuel used in farm machinery. A review of farm tax refund records has begun to reveal this type of information.

1. Data Collection

The initial phase in data collection was the determination of traffic volumes for the roads in the rural compartment. This was accomplished by the use of road tube vehicle counters. These counters were placed on roads in the rural compartment and recorded the number of vehicles passing over the road. Because of the nature of the rural compartment, it was decided that each road in the compartment would be counted. As of the writing of this report, the counting of traffic volumes on the rural compartment roads is nearing completion.

The second phase of data collection will involve a detailed analysis of traffic volumes in the urban compartment. Due to manpower and time limitations, only representative streets will be sampled in this phase. This phase is expected to proceed at a slower rate than that of the rural compartment due to the complex nature of the urban street network. The traffic volume counts taken during the phase will be supplemented by the use of traffic volume maps for the cities of Champaign and Urbana for 1971.

A third phase will be implemented at a later date. This will be the analysis of the additional rural compartment or the "recovery" compartment. This will be accomplished in much the same way as the rural compartment was counted.

Finally, a constant monitoring of key areas will help the team to arrive at seasonal factors to apply to gathered traffic volumes. These factors will attempt to show the seasonal variations in traffic volumes for the study area.

2. Data Analysis

Once the traffic volumes have been collected they are converted into estimates of vehicle miles of travel. These estimates of vehicle miles of travel, and eventually gasoline consumption, will be made for each of the roads in the rural compartment. This analysis will yield a spatial distribution of gasoline consumption and therefore lead output.

At the writing of this report, work has commenced on the analysis of the data from the rural compartment. The analysis has been completed for three areas within the rural compartment:

1) the rural sub-compartment in the northwest corner of the study area;
2) for one-half and one mile radii of the Saline Branch stream gage for the rural compartment; and
3) for one-half and one mile radii of the Brownfield Woods area.

The following table summarizes gasoline consumption for the mentioned areas.

SUMMARY TABLE

Location	Miles of Road	Vehicle Miles (30-day period)	Gasoline Consumption (30-day period) (Gallons)
Rural Sub-Compartment	9.4	44,730	2595
Saline Branch Stream Gage			
1/2-mile radius	2.0	6,420	372
1 -mile radius	5.5	16,170	937
Brownfield Woods			
1/2-mile radius	3.0	23,580	1367
1 -mile radius	6.5	52,170	3022

Work is being done on developing a map showing the gasoline consumption and finally the lead output for each road in the rural compartment. When this analysis is completed, the second phase of data analysis will begin. This phase will involve analysis of data from the urban compartment. As was stated earlier, this phase is expected to proceed more slowly than the first stage.

Constant updating of the material will continue throughout the study in hopes of determining the seasonal variations in lead output for the study area. It should be kept in mind that the summary table lists gasoline consumption for vehicular traffic only. The analysis of farm machinery consumption is still to be done. This analysis will be used to refine the output of the summary table slightly.

3. Rainwater

Other possible lead inputs to the watershed such as airborne lead originating outside the watershed but deposited within the watershed and lead input in rainwater must be considered. Some lead deposition studies have recently begun which will involve the collection of dust-fall in acid-leached, 2-liter, wide-mouthed polyethylene bottles. Several transect studies at right angles to major highways are being established as well as in remote areas (zone 3) of the watershed.

Lead in rainwater has been sampled during several periods of rainfall in March and April. Preliminary sampling was done at four locations in the watershed as listed in Table 10. Results for one 16-hr. period of extended, heavy rainfall are also shown in Table 10. These samples were collected by placing four wide-mouth, acid-leached polyethylene bottles at each location in the late afternoon prior to the start of the rain. Bottles were picked up the following morning after a total rainfall (Urbana measurement) of 1.71 inches.

Table 10

Lead in Rainwater (ppb)

	Within Watershed				Outside Watershed
	Rural		Urban		
	Leverett	Subwatershed	Campus	Sewage Plant	Villa Grove
Avg. Pb	4.0	5.8	2.6	6.9	2.7
Avg.	4.7		4.8		

No significant differences were apparent from the data. Based on the average lead in these samples and an annual rainfall of 35.65 inches, total lead entering the watershed by rainwater was calculated and is shown in Table 11.

Table 11

Lead in Rainwater (12-month period)

Range	Concentration ppb	Total Pb g
Minimum	2.4	5,000
Average	4.7	10,000
Maximum	8.9	19,000

Rainfall falling through the canopy of Brownfield Woods was collected at 48 stations during several periods of rainfall in June, July and August of 1971. Average lead content of these samples was 3.6 ppb. Data revealed no relationship to date or to distance from a roadway. These values compare favorably with rainwater samples discussed above.

Further observations on lead in rainwater are planned. Recording rain gages are being installed at four locations in the watershed, two in the rural area and two in the urban area. Three wedge-type gages for sample collection will be located around the recording gage at each site. After each rain, samples will be collected for analysis and gages will be flushed with a dilute HCl solution.

B. Output Measures

The primary avenue of exit for lead from the watershed is through stream water, including lead carried with the suspended load. Attention has focused to date on development of sampling technique and equipment for monitoring this movement.

1. Stream Gaging

Surface drainage discharge has been continuously monitored with four stream gaging stations located on the Saline Branch Drainage Ditch and the Boneyard Creek. Total water volume moving past each gage is determined for each 24-hour period from noon to noon. This time period corresponds with the water sampling time period. Locations of the gages are shown on Figure 21, an idealized diagram of the watershed. Gages are operational at all locations shown except the rural sub-compartment gage which is scheduled to be in operation during the summer.

2. Water Sampling

Continuous duty automatic water samplers have been developed to collect water samples at each location. These units have been in operation since March 1.

The major components of the sampler are the peristaltic pump, stream level sensing unit, solenoid activated valves, and a timing control unit. A block diagram is shown in Figure 22. The pump runs continuously, with a steady flow of water taken from the center of the stream, 6 inches below the surface, passing through the valves and then back into the stream. During low stream flows, the low flow sampling circuit is activated and 100 ml samples are collected at 1-hour intervals and composited in a 26-liter acid-leached polyethylene bottle. During high stream flows, the high flow sampling circuit is activated by the sensing unit and 200 ml samples are collected and composited at 12-minute intervals. Subsamples are taken from the composite bottles at 24-hour intervals for analysis.

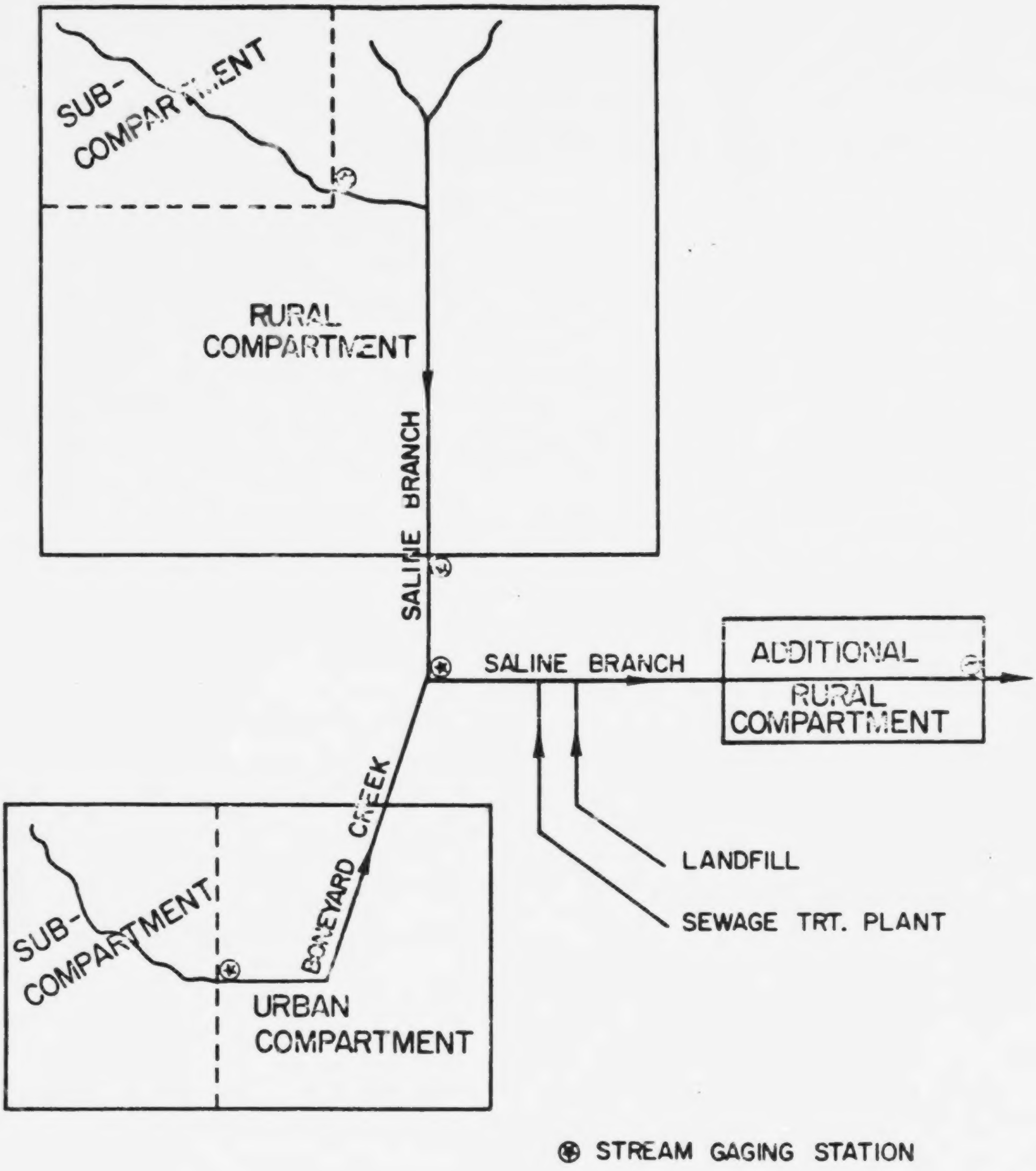
SUB-COMPARTMENT
RURAL COMPARTMENT
SALINE BRANCH
SALINE BRANCH
ADDITIONAL RURAL COMPARTMENT
BONEYARD CREEK
LANDFILL
SEWAGE TRT. PLANT
SUB-COMPARTMENT
URBAN COMPARTMENT
⊛ STREAM GAGING STATION

Figure 21. Schematic Diagram of Idealized Saline Branch Watershed.

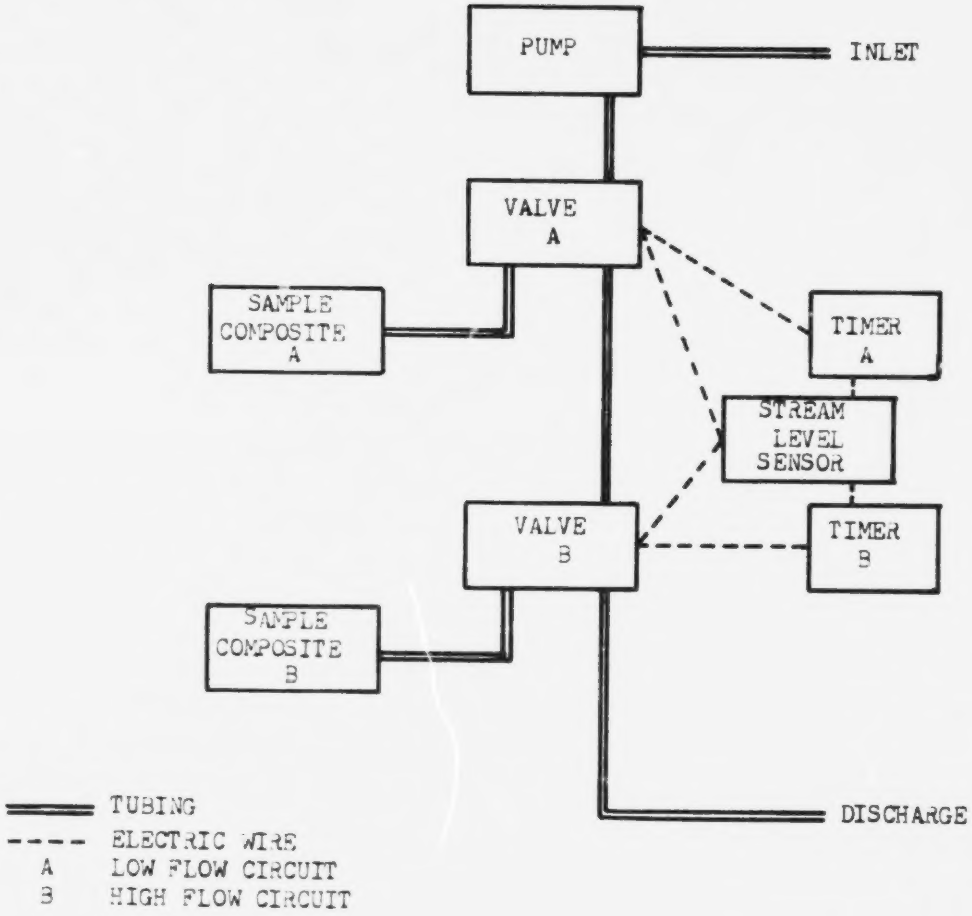
PUMP
INLET
VALVE A
SAMPLE COMPOSITE A
TIMER A
STREAM LEVEL SENSOR
VALVE B
TIMER B
SAMPLE COMPOSITE B
DISCHARGE
TUBING
ELECTRIC WIRE
A LOW FLOW CIRCUIT
B HIGH FLOW CIRCUIT

Figure 22. Block Diagram of Water Sampler

The liquid portion of the water sample is analyzed by anodic stripping voltametry. Concentrations are generally in the 0 to 15 ppb range. Suspended solids in the sample are removed by filtration with an 5-micron millipore filter which through experimentation has been found to remove at least 90% of the solids. Preliminary filtration work was done with .45-micron millipore filters but premature plugging of the filters occurred. The validity of using the 5-micron filter is presently being evaluated. Suspended solids results are reported as total micrograms of lead per liter of sample.

3. Results

Preliminary results are available for the month of March. Typical results for a five-day period in late March are shown in Tables 12 and 13 on a 24-hour basis. Total lead output in grams for the rural area of the watershed for a 30-day period is shown in Table 12 and from the urban area in Table 13. The five-day period shown in Tables 12 and 13 represents a typical spring storm period occurring when the stream level is above normal. Total lead output from the urban area for March is approximately twice the total lead output from the rural area. The ratio of lead output urban to lead output rural is expected to be even greater during the summer when rains are more infrequent. When a rain occurs, there should be a flushing of lead from the extensive area of paved surfaces in the urban area which should result in a wider ratio during the storm period.

4. Other Outputs

Other possible losses of lead from the watershed include lead in air and lead in grain or livestock. Initially, lead moving out of the watershed in the air is assumed to be equal to lead moving into the watershed in the air. Lead in livestock exported is expected to be negligible since livestock is a very minor component of agricultural industries within the watershed.

Table 12. LEAD MOVEMENT FROM RURAL SECTION OF WATERSHED (24-Hour Basis)

Date	Volume liter x 10^7	Pb(Solids) g	Pb(Liquid) g	Total Pb g
3-22	3.50	70	53	123
3-23	4.47	161	94	255
3-24	3.73	67	54	121
3-25	3.49	63	38	101
3-26	3.41	61	34	96

Rural 30-day total = 4600 g

Table 13. LEAD MOVEMENT FROM TOTAL ECOSYSTEM

SEWAGE TREATMENT PLANT

Date	Volume liter x 10^7	Pb(Solids) g	Pb(Liquid) g	Total Pb g
3-22	9.30	237	131	368
3-23	13.00	624	208	832
3-24	10.50	315	210	525
3-25	9.57	249	134	383
3-26	9.23	222	138	360

30-day Total	15,750 g
Rural Contribution	-4,600 g
Urban Contribution	11,150 g

Table 14

Lead Inputs

(30 days)

Gasoline Consumption	3,648,000 g
Rainwater (Pb)	1,600 g

Lead Outputs

(30 days)

Stream Water	16,000 g
Harvest (Corn)	< 100 g (Year)
(Soybeans)	< 75 g (Year)

Due to the large amounts of corn and soybeans exported from the primarily agricultural ecosystem, an intensive sampling of these crop plants was completed in the fall of 1971. In the majority of cases, all of the stover is left in the field except for a minor export of cobs. An estimated 2,328,000 bushels of corn were shipped from the watershed in 1971. Evaluation of the soybean data is incomplete at this time. Results of the laboratory analysis of corn grain, cob, and stover are complete and indicate an average lead content in grain (beyond 50 m from highways) of 0.1 ppm. No significant difference was found between grain and cob. The stover averaged 8.0 ppm. No significant trends in relation to distance from a highway were found in these samples, all of which were collected at distances greater than 50 m from the nearest road (Zone 3).

Based on reported corn yields in Champaign County for 1971, an estimate of lead removed from the watershed in exported corn grain was calculated using the average grain lead level. This estimate is a rather insignificant 86 grams. It is expected that soybean data will be quite comparable with corn.

Further samples of corn, soybeans, and wheat will be collected during 1972 to complete estimates of lead export in this produce.

Table 14 is a summary of present input and output values for lead in the watershed.

C. Soil-Plant Sampling

Sampling for lead in soil is continuing. Intensive sampling of the rural subwatershed is complete. Composite samples of the 0 to 10 cm depth were taken at 50 m intervals on 2 north-south transects and 2 east-west transects through each section of the subwatershed. Data are presented in Figure 23 . Further sampling is underway to complete background information on lead distribution as a function of distance from highways, traffic volumes, and depth in the soil profile. Although analysis of the several hundred samples taken this spring is incomplete, the limited data available reveal no unusual results. Steep gradients away from highways, sharper on the upwind side, are present with levels near the pavement ranging from 20 to 200 ppm depending on traffic volume levels in Zone 3, remote from all roads, average 16 ppm. This phase of the study will be completed by July 1972.

Only plant lead data from earlier studies (already reported) are available. Major efforts during the summer will be directed to obtaining early fall estimates of plant biomass and lead content in all compartments of the ecosystem. Peak standing crop will also be used for many non-agricultural species of importance in the watershed. Crops will be sampled more frequently.

In all sampling, emphasis will be placed on the portion of the plant consumed by herbivores or harvested for export. Close coordination is expected between plant and animal sampling groups, in order to maximize foodchain information.

D. Small Mammal Sampling

1. Introduction

The initial studies of lead uptake by vertebrates was restricted to the various species of small mammals living in the study region. Small mammals were selected because their ranges of activity are somewhat restricted and would provide a better opportunity to evaluate the concentration

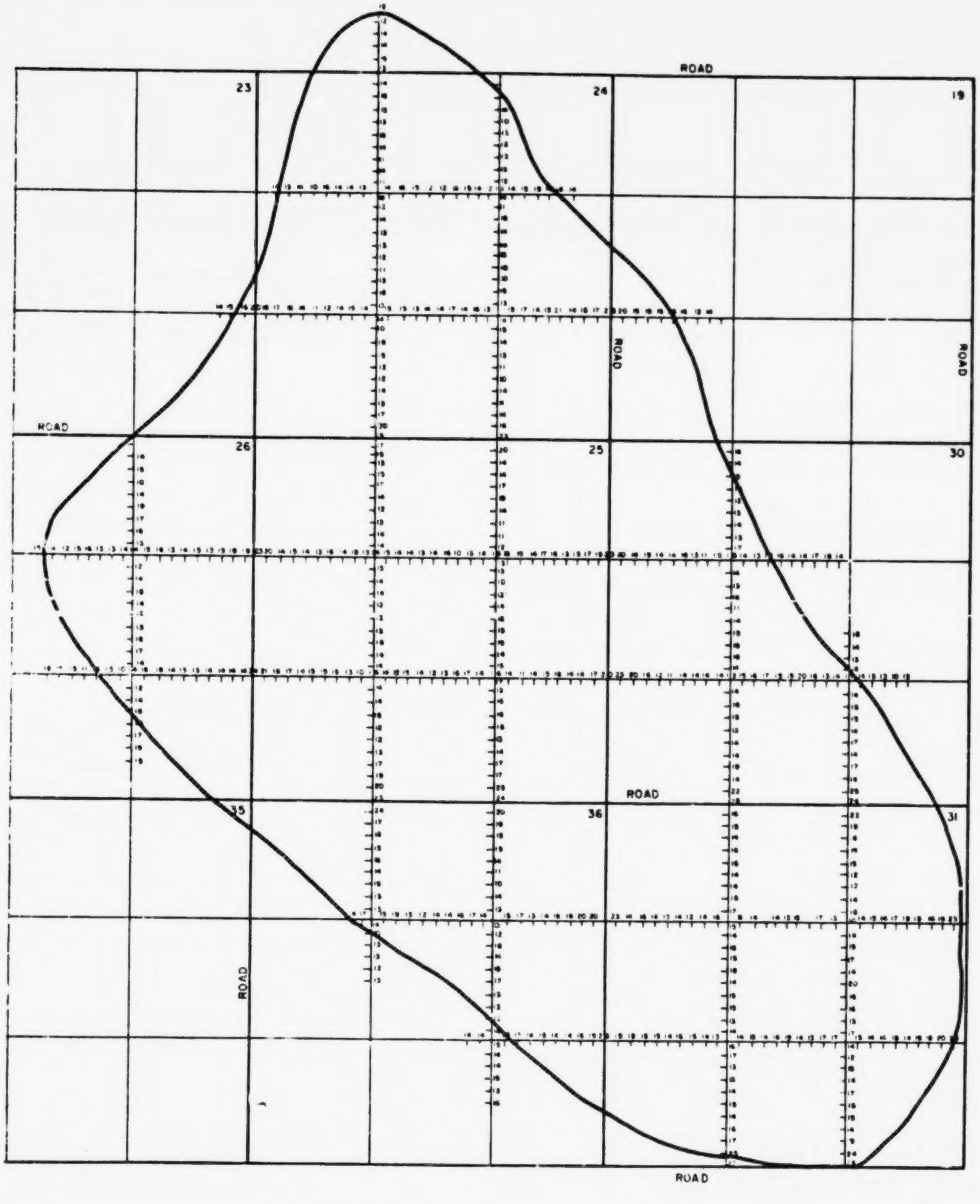
ROAD
23
24
19
ROAD
ROAD
ROAD
26
25
30
ROAD
35
36
31
ROAD
ROAD

Figure 23. Soil Lead Concentration (ppm)
in the Rural Subwatershed.

of lead in animals living within the sphere of influence of roads as contrasted to those occupying habitats remote from areas exposed to automobile exhaust lead. Birds and larger mammals range over too wide an area and would not show a close enough association to highways to accurately evaluate the percentage of time they were living in sites with high environmental lead input. The home ranges of the small mammals studied (with the exception; see below) were small enough that those individuals trapped adjacent to the roads could be considered to have been living entirely within the area influenced by the highway.

2. Methods

Small mammals were obtained by snap-trapping. A variety of situations were selected to give a broad coverage of the various possible levels of lead input into the environment. These included areas adjacent to heavy-use roads such as interstates (trapping was done within 5 m of the road), adjacent to secondary roads estimated to have very little traffic (trapping was done within 5 m of these roads), and areas away from the influence of roads of any type (most of these sites were at least 100 m away from a road). The latter sites away from roads were selected to include both natural habitats as well as those representing the common agricultural crop habitats within the study region.

In addition to obtaining animals to be used in determining the actual concentration of lead within the animal tissues, other study sites were established to estimate population densities in the various situations listed above. Live-trapping is being done in order to obtain an estimate of the total biomass in each habitat type (and therefore in the entire study region) so as to be able to determine the total amount of lead in residency within the mammal tissue at any given time. These studies are being conducted on a seasonal basis to estimate the seasonal and annual fluctuations in densities of the various species within the study region. This in turn will permit estimates of the fluctuation in the total biomass of small mammals (and thus lead in residency in the small mammals) in the study region.

3. Results

Preliminary analyses were made of the lead content of specimens of four common species obtained in the winter trapping. Comparisons were made of five animals, each of the four species from an area adjacent to an interstate and from situations at least 200 m away from any roads. The results of these preliminary analyses are being used to further refine the techniques to be used in processing the remaining specimens.

The results of the preliminary lead analysis are shown in Table 15. In brief, these show that the house mice, *Mus musculus*, living adjacent to the high-use highway, have approximately three times as much lead as do those individuals living away from the influence of roads. The harvest mice, *Reithrodontomys megalotis*, along the roads contain almost five times as much lead as do those living away from roads. The short-tailed shrews, *Blarina brevicauda*, living along the high-use road, have approximately five times as much lead as do those individuals living away from roads. The prairie deermice, *Peromyscus maniculatus*, along the high-use highway, have only slightly more lead than do those individuals living away from roads. This latter species has a much larger home range than do the other species, and also does not show as close a restriction to areas of dense vegetation as do the other species. The prairie deermouse makes extensive use of open field situations and occurs only incidentally in areas of high density vegetation such as occur along roadsides. The individuals captured along

Table 15

Lead Concentration in Small Mammals from Sites Within 10 m of A Heavy-Use Highway and Sites 200 m away from Roads (5 Individuals of each Species from each Site)

Species	Lead Content (ppm, dry weight)	
	Adjacent to road	Away from road
Mus musculus	15.4 (8.0-25.0)	5.3 (3.0-8.5)
Reithrodontomys megalotis	20.8 (14.0-35.0)	4.2 (3.8-5.3)
Peromyscus maniculatus	11.0 (7.0-19.0)	7.8 (3.6-10.0)
Blarina brevicauda	21.2 (12.0-36.0)	4.4 (3.8-5.0)
Average	17.1	5.4

the road may represent mice that had home ranges extending far out into the fields and thus were not spending a significant portion of their time in a site with high lead input.

The projected work for the coming summer and fall will concentrate on determining the location of lead in the specimens, in the tissues, in the digestive track, or on the hair. We will also process the remaining specimens we obtained during the winter trapping so as to get statistically significant data concerning lead concentration in the varied species in the different situations discussed above. We will also do more detailed sampling to determine the actual relationship of the individuals trapped to the zones of high lead input adjacent to the roadways. We will also continue the studies of population densities and fluctuations within the study region so as to obtain adequate estimates of fluctuations in population densities on a seasonal and annual basis.

E. Invertebrate Work

The invertebrate work was not started during the winter owing to very low densities of invertebrates during this season. Preliminary collecting has now been started. Samples will be available for processing by the first of June. The main work concerning invertebrate consumers will be done during the summer when such species are more abundant and adequately sized samples for lead determination will be available.

F. Aquatic Communities

1. Introduction

Fish, benthos, stream drift, periphyton, and rooted plants constitute the aquatic communities considered. Plankton is being ignored because of its insignificance in this stream system. The approach to this investigation consisted of two parts: first, to determine the lead levels in various organisms constituting the aquatic communities and, second, to estimate the biomass of the various community components. During the last half of February and March, most of the effort went into collecting large numbers of organisms and preparing them for analysis of lead levels. Little attention was given to the actual abundance of these organisms in the community until April when an estimate was made of the actual weights and numbers of organisms present in the different compartments of the stream community.

2. Sampling

Many technical problems have been encountered and most of them solved as the aquatic communities have been sampled and analyzed. Actual collecting and sampling involved both quantitative and qualitative measures of the fish, benthos, drift, and aquatic plants. One of the first problems encountered was to determine the sample size for reliable lead analyses. Fish are collected using two seines concurrently in a measured sampling area. A rather uniform stream habitat permitted this method to be used rather efficiently for a quantitative estimate of the problem. Benthic organisms are procured either with a hand-operated grab or a piston-coring device, the choice depending upon the bottom material being sampled. The total communities will be calculated from the observed abundance in various bottom types and the amount of each bottom type in the stream system. Stream drift is collected by straining a measured volume of water through a fine-mesh net. Aquatic plants are collected simply by removing all the plants found in a measured area of the stream.

Periphyton, or Aufwuchs, constitutes the greatest biomass produced in the stream and the most dynamic biological component. To efficiently permit measurements of production, lead accumulation, and export of Aufwuchs, an artificial substrate is placed at several locations in the stream. The most satisfactory substrate tested is a 64-ounce polyethylene bottle filled with water for stability and anchored to the stream bed. The sampler is removed at 12-14 days after immersion and accumulated growth is scraped off.

Measurements of sloughing (export) of Aufwuchs and associated lead are made using portions of the composite water samples obtained daily. Separation is made by filtration.

3. Results

At this time, only incomplete data on the community biomass and lead levels in each compartment of the ecosystem are available. Invertebrate and fish populations appear to be higher (Tables 16-19) in the rural subcompartment than farther downstream in the main rural compartment where the bottom materials are less stable. The small number of lead determinations completed (Table 20) must be reviewed cautiously. Tubificids have accumulated rather great amounts of lead below the urban compartment. Chironomid larvae likewise may contain substantial levels. Fish of various species generally carry 3 to 10 ppm of lead and showed no consistent differences above or below the major input of lead.

Although many new problems are being uncovered as the work progresses, the field and laboratory methods have been worked out, many samples are ready for analysis, and soon total lead accumulations in the aquatic communities can be calculated.

Table 16.

Aquatic Invertebrates in the Saline Branch of the Rural Subcompartment taken in April 1972

Organism	Wet weight/area (g/m^2)				
	Sample 1	Sample 2	Sample 3	Sample 4	Mean
Chironominae	0.404	0.084	0.127	0.243	0.214
Tubificidae	11.542	12.921	3.151	6.249	8.477
Hirudinea	32.211	11.989	15.802	22.618	20.654
Dubiraphria	0.029		0.013		0.011
Ceratopogonidae	0.053		0.024	0.019	0.024
Physa	1.108	0.684			0.448
Sphaeriidae	3.342	0.363	2.184	1.288	1.794
Zygoptera			1.065		0.265
All others			0.175	0.196	0.093
Total	48.734	26.042	22.541	30.613	31.983
% Analyzed for Lead	99.94	100.00	99.05	99.29	99.60

Table 17.
Aquatic Invertebrates in the Saline Branch of the Rural Compartment taken in April 1972

	Wet weight/area (g/meter^2)			
Organism	Sample 1	Sample 2	Sample 3	Mean
Chironominae	0.201	0.574	0.095	0.290
Tubificidae	0.300	8.037	2.167	3.501
Ceratopogonidae	0.040	0.026	0.071	0.046
Dubiraphria	0.019	0.045		0.021
Hexagenia	6.468	2.696	0.315	3.160
Caenis	0.093	0.042		0.045
Ephemerella		0.074		0.025
Zygoptera		0.003		0.001
Oligochaeta			7.413	2.471
All others	0.056	1.172	0.053	0.427
Total	7.124	12.669	10.114	
% Analyzed for Lead	98.38	90.18	98.77	95.05

Table 18.

Fish taken from a Tributary to Saline Ditch in the Rural Subcompartment in April 1972

Fish	Wet weight/area (g/meter^2)			
	Sample 1	Sample 2	Sample 3	Mean
Erimyzon oblongus	0.299	0.052	0.057	0.136
Campostoma anomalum		0.023		0.008
Ericymba buccata	2.412	0.745	1.527	1.561
Notatus umbratilus		0.035	0.076	0.037
Pimephales notatus	0.971	0.508	0.773	0.751
Semotilus atromaculatus	12.56		0.140	4.233
Lepomis cyanellis			0.014	0.005
Etheostoma nigrum	0.014		0.012	0.009
Total weight	16.256	1.336	2.599	6.730

Table 19.
Fish Taken from the Saline Ditch (Urbana Gage Site) in the
Rural Compartment, April 1972

Fish	Wet weight (g/m^2)				g/acre-ft
	Sample 1	Sample 2	Sample 3	Mean	
Carpiodes sp.	1.000	0.832	0.038	0.623	1851.1
Cyprinus carpio	0.675			0.225	777.5
Campostoma anomalum		0.049		0.016	39.9
Ericymba buccata	0.006	0.027	0.001	0.011	29.7
Notemigonus crysoleucas	0.006			0.002	6.5
Notropis chrysocephalus	0.021	0.022	0.003	0.015	44.5
Notropis stramineus	0.001	0.009		0.004	9.2
Notropis umbratilus	0.055	0.076	0.110	0.080	201.2
Pimephales notatus	0.022	0.498	0.009	0.176	434.7
Semotilus atromaculatus	0.011	0.005		0.005	16.0
Etheostoma nigrum	0.002			0.001	1.8
Total weight	1.799	1.518	0.16	1.158	

Table 20.

Mean Value of Lead (ppm dry tissue) and Number of Analyses (in parentheses) of Aquatic Organisms from the Saline Ditch, March 1972

	Rural sub-compartment	Rural compartment	Urbana compartment	Additional compartment
Tubificidae			50-200 (2)	370 (1)
Chironominae				50 (5)
Hexagenia		20 (1)		
Decapoda		7.5 (5)		10 (1)
FISH				
Notropis stramineus		5.5-6.9 (2)		
Notropis crysoleucas				2.6-3.1 (2)
Notropis umbratilus	2.8-3.5 (3)			
Notropis spilopterus		6.8 (1)		
Notropis chrysocephalus		2.2		
Cyprinus carpio	4.1 (1)			5.3 (1)
Erimyzon oblongus	5.9 (1)			3.8 (1)
Campostoma anomalum				2.7-6.8 (2)
Pimephales notatus	2.9-6.4 (3)	1.5-5.2 (2)		
Catostomus commersoni				1.3 (1)
Ericymba buccata	2.6 (3)	1.8		6.8
Semotilus atromaculatus	1.8-2.3 (2)			
Lepomis cyanellus	16			
PLANT				
Elodea canadensis				290-330 (3)

G. Personnel

Input Measures

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Dr. A. Haney

Output Measures

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Animal Studies

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Aquatic Studies

Dr. R. Larimore
J. McNurney
Dr. H. Leland
J. Baker

V. AIR-SOIL-WATER-PLANT CONTINUUM

INTRODUCTION

It is the purpose of the Air-Soil-Water-Plant group to study the forms of lead and other trace elements within the continuum, the localization of these forms, and the mechanisms by which lead transfer occurs from one site to another. Such knowledge is critical to virtually all the other groups of the project, since the mechanisms of mobility and localization of lead within the ecosystem should be delineated here. It is the understanding of these mechanisms that will be crucial in predicting shifts in future lead compartmental concentrations within the ecosystem. While the present ecosystem levels of lead are apparently not generally harmful, they could be if localized at certain sites.

The research reports in this section have been divided into groups within the continuum. Such a division is only a reporting convenience, however, and the fact that we are dealing with lead flow within a continuum must not be lost sight of by either the reader or researcher.

A. Aerosol Lead Particles

1. Background

The lead halides, lead bromide ($PbBr_2$), lead chloride ($PbCl_2$), lead bromochloride (PbBrCl), and the alpha and beta forms of the double salt lead bromochloride ammonium chloride ($2PbBrCl \cdot NH_4Cl$) account for 80 to 100% of the lead containing compounds found in the exhaust of automobiles burning gasoline containing a lead antiknock additive with ethylene dibromide and ethylene dichloride lead scavengers (Habibi, et al., 1970; Hirschler, et al., 1957; Bayard and Ter Haar, 1971). The exact composition ratio of exhausted lead compounds depends on driving conditions and the fuel used. After being exhausted from the automobile, these lead halides can lose free halogen by photochemical decomposition due to irradiation by ultraviolet light from the sun (Pierrard, 1969). In addition, it is also known that freshly exhausted particulates readily lose halogen even in the absence of sunlight (Bayard and Ter Harr, 1971; Robbins and Snitz, 1972). There is some evidence (Bayard and Ter Haar, 1971) that this halogen loss occurs, presumably, as the halide ion by hydrolytic exchange with water and carbon dioxide in the atmosphere to form basic lead halides and basic lead carbonates.

Numerous studies have been conducted to determine the particle size distribution of automobile exhaust particulates (Habibi, et al., 1970; Hirschler, et al., 1957; Hirschler, et al., 1964; Mueller, et al., 1962). Although particle size distribution varies with engine operating conditions, from 20 to 30% by weight is exhausted as particles in the greater than 5 micron diameter range; from 50 to 70% in the 1 to 5 micron range; and 5% or less in the submicron range (Hirschler, et al., 1957; Hirschler, et al., 1964). However, in terms of particle count, greater than 90% of exhaust particles have a diameter less than 2 microns (Hirschler, et al., 1957; Mueller, et al., 1962). Because particles larger than 1 to 2 microns quickly settle to the ground, the mass median diameter of airborne lead containing particles is about 0.25 microns (Bayard and Ter Haar, 1971; Mueller, et al., 1962).

According to Habibi, et al. (1970), PbBrCl is the major lead salt in particles of 2 to 10 microns equivalent diameter with $2PbBrCl \cdot NH_4Cl$ being present as a minor constituent, while the submicron lead-containing exhaust particles are primarily $2PbBrCl \cdot NH_4Cl$.

In order to simulate the reactions and transport of automobile exhaust particulates with laboratory pure compounds, the pure compounds should have a morphology and particle size distribution as similar to the exhaust particles found in the environment as possible. In this investigation aerosols of $PbBr_2$ and PbBrCl were produced from controlled temperature melts, as described in the experimental section, and were studied by transmission and scanning electron microscopy. In addition the effect on the aerosols of irradiation by ultraviolet light and exposure to high humidity, high carbon dioxide content atmospheres was investigated to determine if the laboratory pure lead halides would undergo loss of halogen similar to that reported for automobile exhaust particulates.

2. Procedures

Lead bromide and lead bromochloride aerosols were intially produced from controlled temperature melts of the pure compounds as illustrated in Figure 24. By adjusting the voltage applied to the chromel heating coil with a Variac variable transformer, the melt temperature was kept at 400°C for $PbBr_2$ (melting point 373°C) and 450°C for

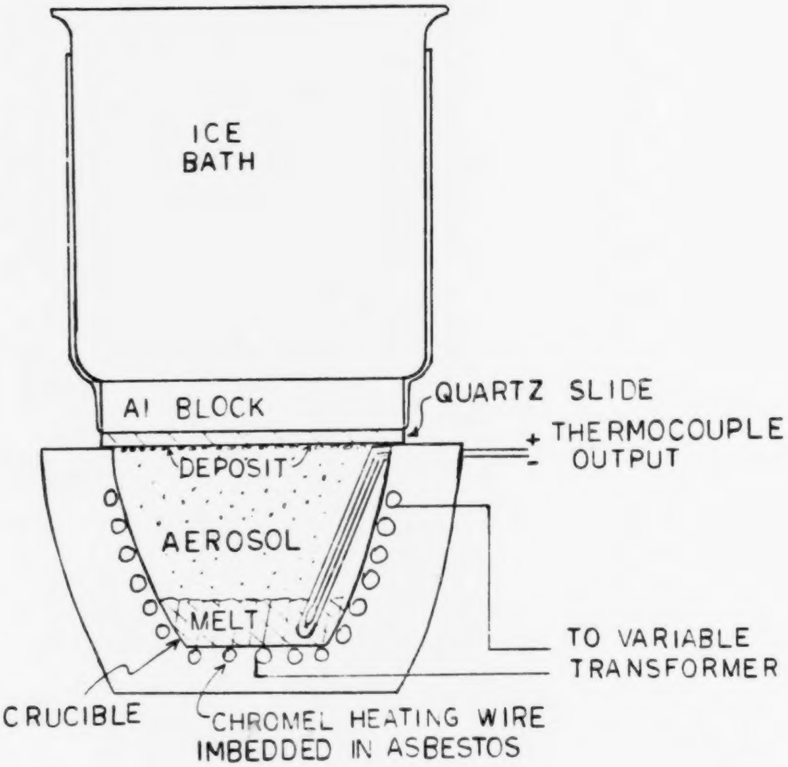
ICE
BATH
Al BLOCK
QUARTZ SLIDE
+
THERMOCOUPLE
-
OUTPUT
DEPOSIT
AEROSOL
MELT
TO VARIABLE
TRANSFORMER
CRUCIBLE
CHROMEL HEATING WIRE
IMBEDDED IN ASBESTOS

Figure 24. Apparatus for collection of lead halide aerosol particles from controlled temperature melts.

PbBrCl (melting point 430°C) as read by calibrated iron-constantan thermocouple. At these temperatures the vapor pressure of the two lead halides was sufficient to produce a visible white "smoke" above the melt.

As illustrated in Figure 24 lead halide "smoke" particles were allowed to collect on a 90 mm diameter quartz slide for study by electron microscopy and X-ray diffraction. That $PbBr_2$ and PbBrCl were actually the materials being collected was verified by comparison with literature values of the interplaner spacings and line intensities obtained by X-ray diffraction.

Both lead bromide and lead bromochloride aerosols were collected on quartz slides, as described above, and placed in a Pyrex chamber with optically flat faces inside a constant temperature control box. Here the samples were irradiated in dry CO_2 free air or nitrogen with ultraviolet light from two Black-Lite B-100A lamps at an intensity of 6.3 milliwatts/cm^2 between 3000 Å and 4000 Å, or were exposed without being irradiated to air with a high carbon dioxide, high humidity content. The constant temperature control box was capable of ± 2°C regulation between 20°C and 100°C. The carbon dioxide level was controlled by controlling the air and carbon dioxide flow rates into the Pyrex chamber and the humidity was controlled by saturating the air with water vapor at a known temperature.

Halide ion lost during exposure to high CO_2, high humidity conditions was captured by passing the sweep gas into $0.05\underline{N}$ $NaHCO_3$/$0.05\underline{N}$ Na_2CO_3 buffer. After exposure to the high CO_2, high humidity atmosphere was stopped, the total halide ion captured was determined by coulometric titration with silver ion using a biamperometric endpoint. No attempt was made to determine free halogen lost during exposure of PbBrCl to high CO_2, high humidity conditions.

Free halogen lost during photodecomposition was determined by passing the sweep gas containing the free halogen into standard methyl orange solutions at pH 2.1. The total free halogen was determined from a working curve of the absorbance change of the methyl orange solutions at 505 nm, while the individual amounts of Br_2 and Cl_2 were determined from a working curve of the absorbance change of the methyl orange solutions at 317 nm (Laitinen and Boyer, 1972). During the photodecomposition studies no attempt was made to determine halogen lost as halide ion.

The total halide ion remaining in the PbBrCl samples was again determined by coulometric titration with silver ion. The total lead ion was determined by atomic absorption spectroscopy.

First stage carbon replicas of the lead bromide aerosol particles were studied by transmission electron microscopy. For the replica studies, the quartz slide in Figure 24 was replaced with an asbestos mat containing a 6.7 cm x 1.8 cm opening at a distance of 7.0 cm from the surface of the melt to expose a 1 inch x 3 inch glass microscope slide to the lead bromide "smoke". The back of the slide was cooled with the ice-water bath as in Figure 24. First stage carbon replicas of the lead bromide particles, which collected on the glass slide, were produced by evaporating a carbon film onto the particles in an RCA vacuum evaporator. The carbon film was scored and individual squares of carbon containing lead bromide particulates were floated off the slide in a dilute solution of nitric acid to which a small amount of ethanol had been added to reduce surface tension. The dilute nitric acid dissolved the lead bromide and left the carbon film replicas, which were picked out of solution with copper 200 mesh transmission microscope sample grids. Electron micrographs of the replicas were obtained with the RCA 3E transmission electron microscope using a 50 KEV accelerating potential.

One disadvantage of the generation scheme in Figure 24 is that the mass rate of aerosol collection is quite low - only about 15 mg/hr. To increase the collection rate a nitrogen stream was used to sweep the lead halide aerosol away from above the melt as shown in Figure 24 . With a 400 ml/min nitrogen flow rate, a PbBrCl melt temperature of 450°C and a reaction chamber temperature of 450°C, the mass collection rate was increased to 95 mg/hr for PbBrCl.

The reaction chamber in Figure 25 was constructed with several inlet ports for the purpose of studying gas phase reactions when more than one aerosol has been introduced into the chamber. We hope to produce $2PbBrCl \cdot NH_4Cl$ aerosols, but this has not been attempted yet.

Electron micrographs of PbBrCl were obtained before and after exposure to ultraviolet light and high humidity, high carbon dioxide conditions with a Cambridge Stereoscan scanning electron microscope using a 30 KEV accelerating potential. To prevent the aerosol particles from "charging-up" when examined under the electron beam they were coated with a thin layer of carbon in an RCA vacuum evaporator.

3. Results and Discussion

a. Electron Micrographs The morphology and approximate size distribution of the laboratory pure lead halide aerosols was determined from electron micrographs.

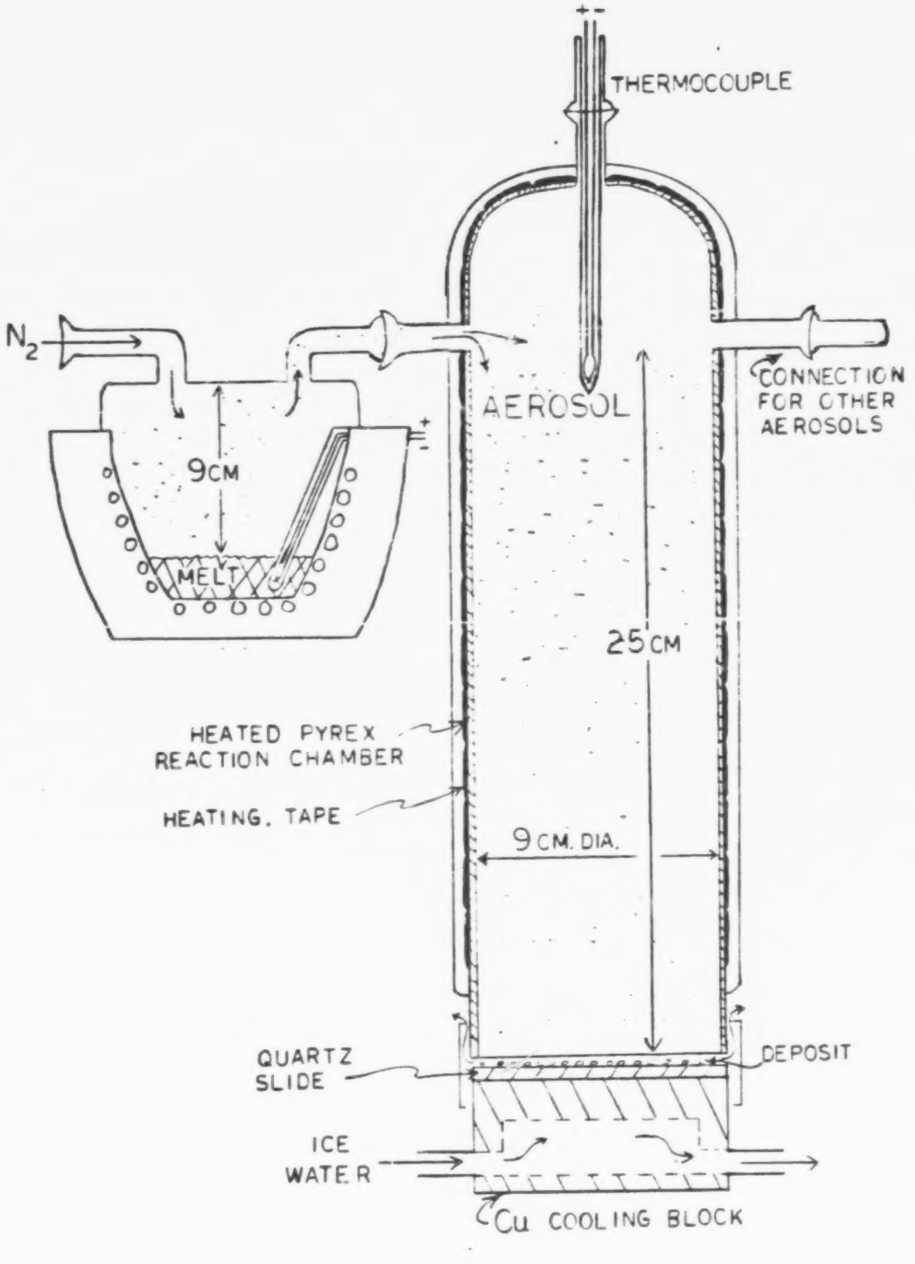
+ -
THERMOCOUPLE
N2
9CM
MELT
AEROSOL
CONNECTION FOR OTHER AEROSOLS
25CM
HEATED PYREX REACTION CHAMBER
HEATING. TAPE
9 CM. DIA.
QUARTZ SLIDE
DEPOSIT
ICE WATER
Cu COOLING BLOCK

Figure 25. Apparatus for reaction of lead halide aerosols with other aerosols.

Figures 26 and 27 are transmission electron micrographs of $PbBr_2$ particles collected on carbon films at 1 and 7 cm, respectively, above the melt. Figure 26 shows that the great majority of the particles collected 1 cm above the melt are spherical particles less than 0.1 micron in diameter. The smallest particles that can be seen in Figure 26 are estimated to be on the order of 50 Å. Of the particles greater than 0.1 micron, 79% are between 0.1 and 0.3 microns in diameter. The mean particle diameter in Figure 27 is 0.36 microns, while the median diameter is 0.25 microns.

Figures 28 and 29 are transmission electron micrographs of carbon replicas of $PbBr_2$ particles collected on a glass slide following the experimental scheme of Figure 24. Figure 28 shows that there are two modes of deposition of $PbBr_2$ on the glass, namely spheres and well defined single crystals. The mean diameter of the spherical particles in Figure 28 is 0.63 microns, while the median diameter is 0.57 microns. Figure 29 shows that some spheres have a surface roughness which suggests that they were formed by agglomeration of smaller particles.

Figure 30 is a scanning electron micrograph of PbBrCl collected on a quartz slide following the experimental scheme of Figure 24. Although it is difficult to determine the mean diameter of PbBrCl particles in this micrograph because the particles are not well separated, the mean diameter appears to be about 0.6 microns. Figures 31 and 32 show the effects of irradiation by ultra-violet light of the PbBrCl in dry, CO_2 free nitrogen and dry, CO_2 free air respectively. These micrographs, which are of the samples whose halogen losses are plotted in Figure 43, show that irradiation by ultraviolet light introduces a fibrous substructure. Some of the resulting needle-like crystals are less than 0.1

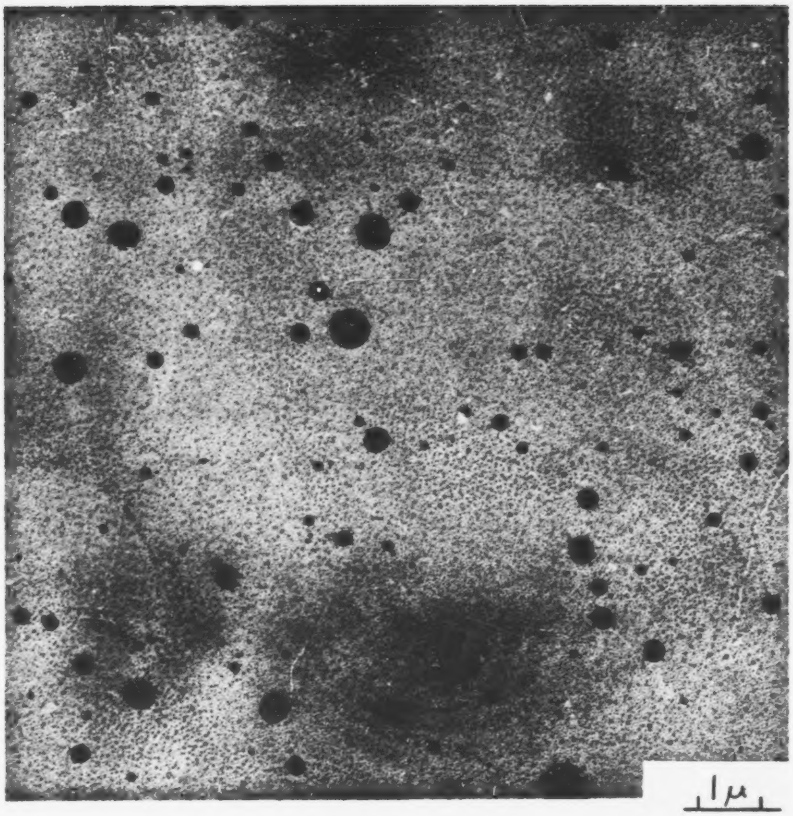

Figure 26. Lead Bromide particles collected on carbon film 1 cm. from surface of melt.

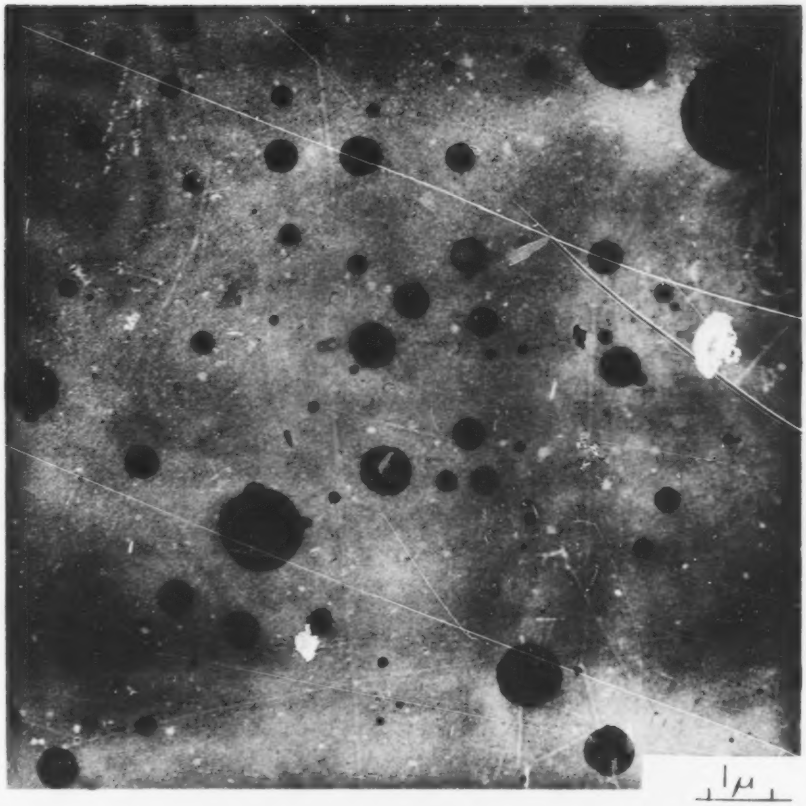

Figure 27. Lead Bromide particles collected on carbon film 7 cm from surface of melt.

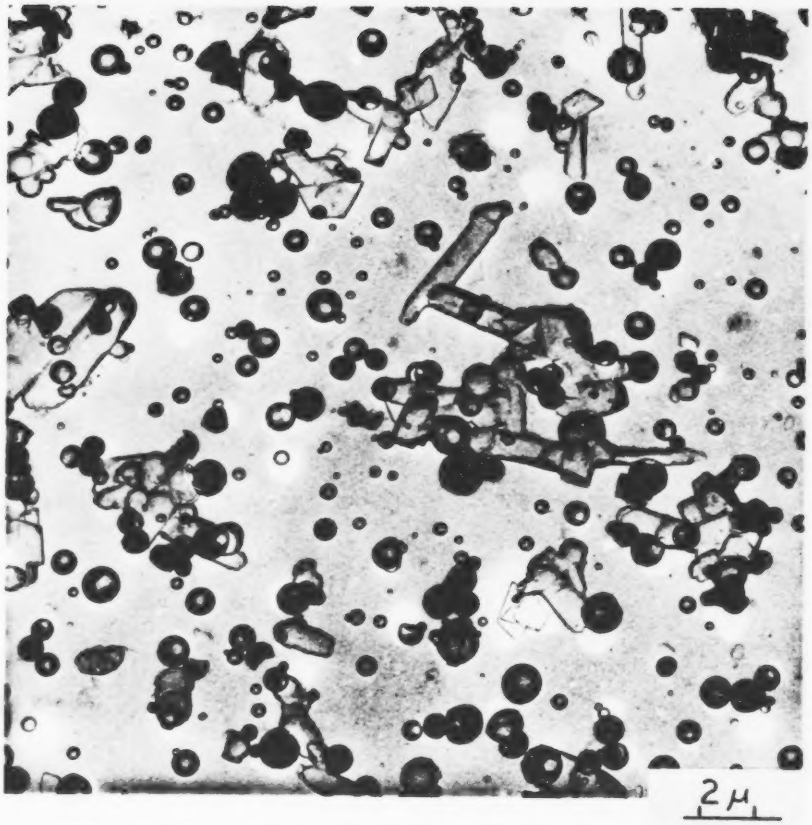

Figure 28. Carbon replicas of $PbBr_2$ particles.

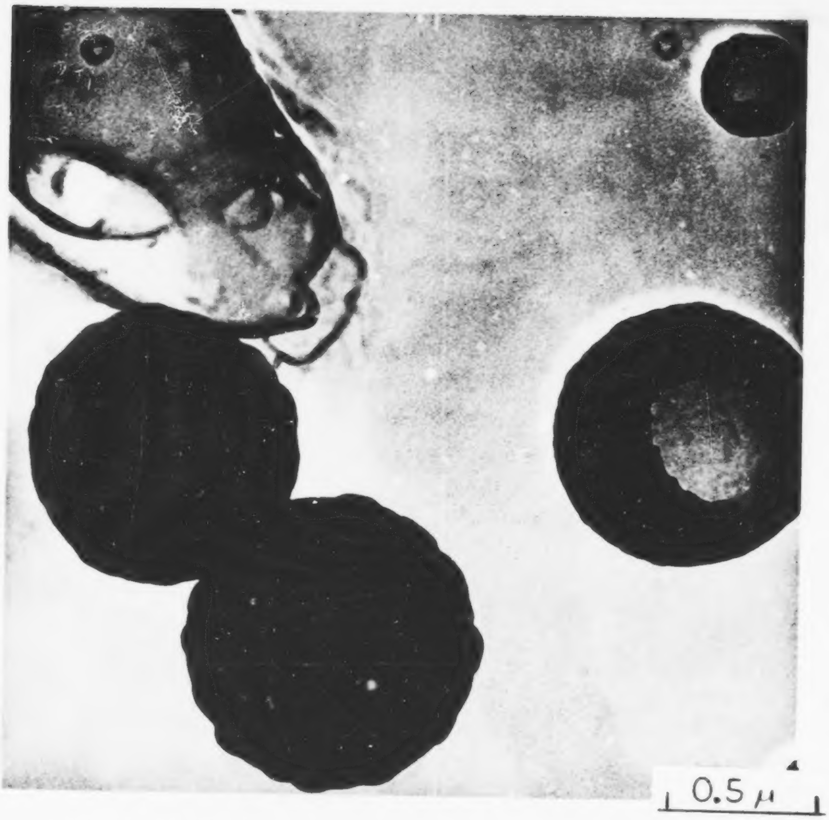

Figure 29. Higher magnification of $PbBr_2$ carbon replicas (shadowed with gold).

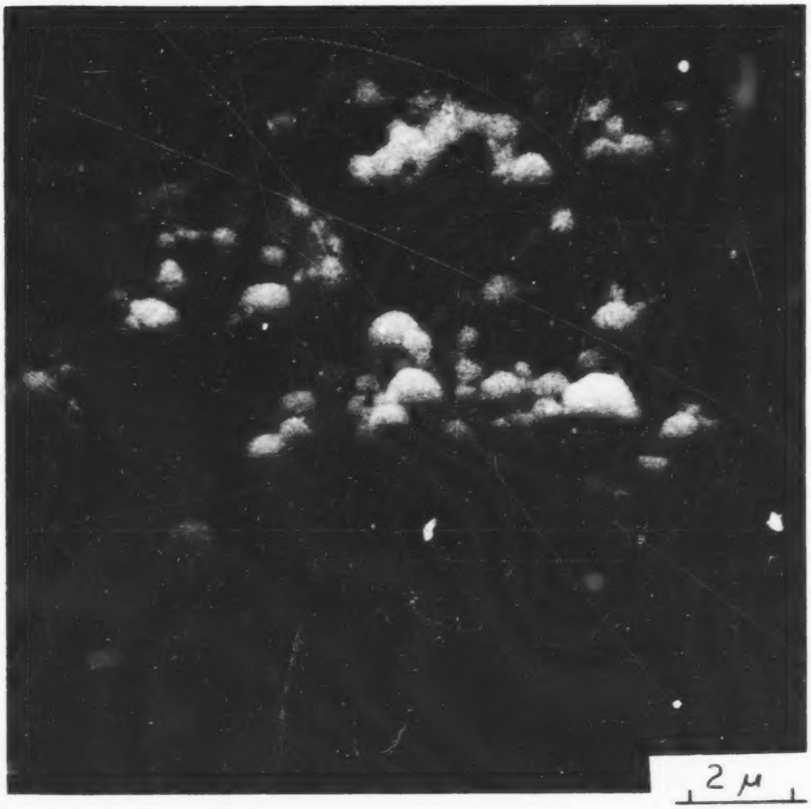

Figure 30. PbBrCl particles collected on quartz using experimental scheme of Figure 24.

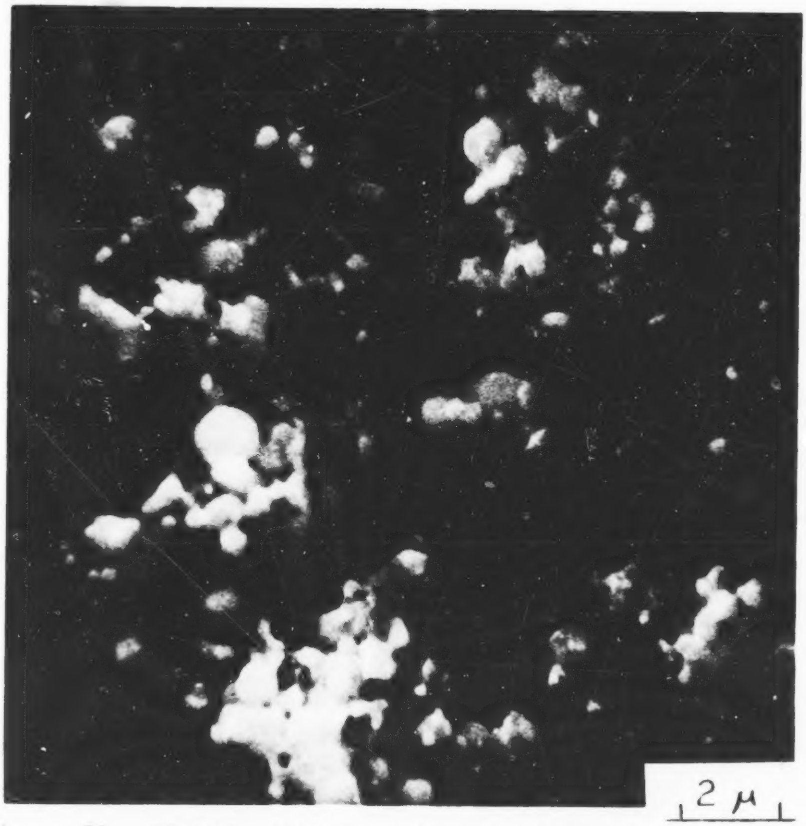

Figure 31. PbBrCl similar to that in Figure 30 after photodecomposition in dry, CO_2 free nitrogen.

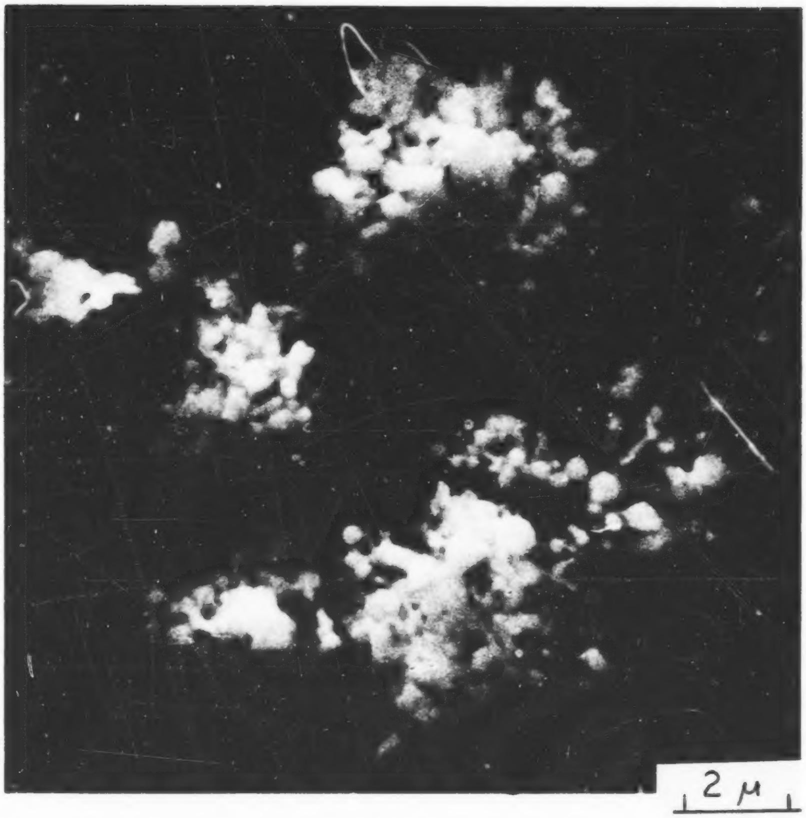

Figure 32. PbBrCl similar to that in Figure 30 after photodecomposition in dry, CO_2 free air.

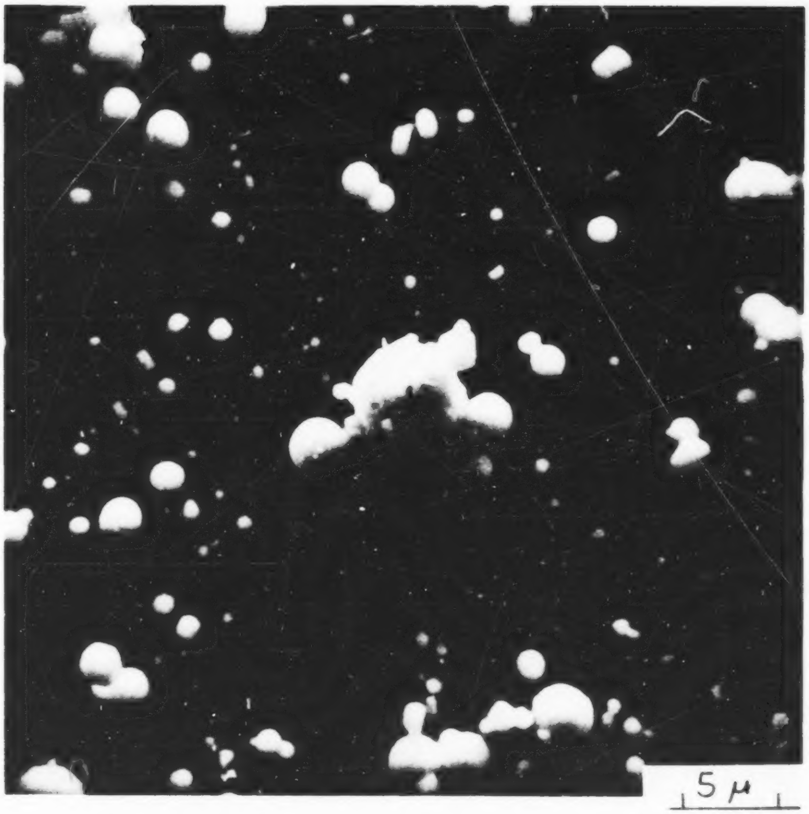

Figure 33. PbBrCl particles caught on carbon film at entrance of reaction chamber in Figure 25.

micron in width. It is planned to determine the composition of these crystals by selected area transmission electron diffraction, but this has not been attempted yet.

Figure 33 is a scanning electron micrograph of PbBrCl aerosol particles caught on a carbon film at the aerosol jet exiting the heated crucible with the reaction chamber in Figure 25 removed. The mean particle diameter in this micrograph is about 2 microns.

Figures 34 and 35 are scanning electron micrographs of PbBrCl aerosol particles collected on a quartz slide using the experimental scheme shown in Figure 25. The mean particle diameter in these micrographs is 3.5 microns.

Figures 36 , 37 and 38 are higher magnifications of particles collected in a manner similar to those in the previous two figures. These micrographs show that the surface morphology varies considerably from particle to particle, ranging from smooth to quite rough. The flat appearance of the left edge of the spheres in Figures 37 and 38 is an artifact introduced by vacuum evaporation of carbon onto the spheres from two different angles to reduce secondary electron emission of the particles and to keep them from charging up under bombardment by the electron beam.

The series of electron micrographs discussed up to now shows that the mean diameter of the aerosol particles increases the farther the aerosol travels in the concentrated form before being dispersed or collected. The formation of particles with distinctly different surface roughness characteristics suggests that the larger aerosol particles may grow by preferential agglomeration of smaller particles having specific electrical charge characteristics. The static electrical

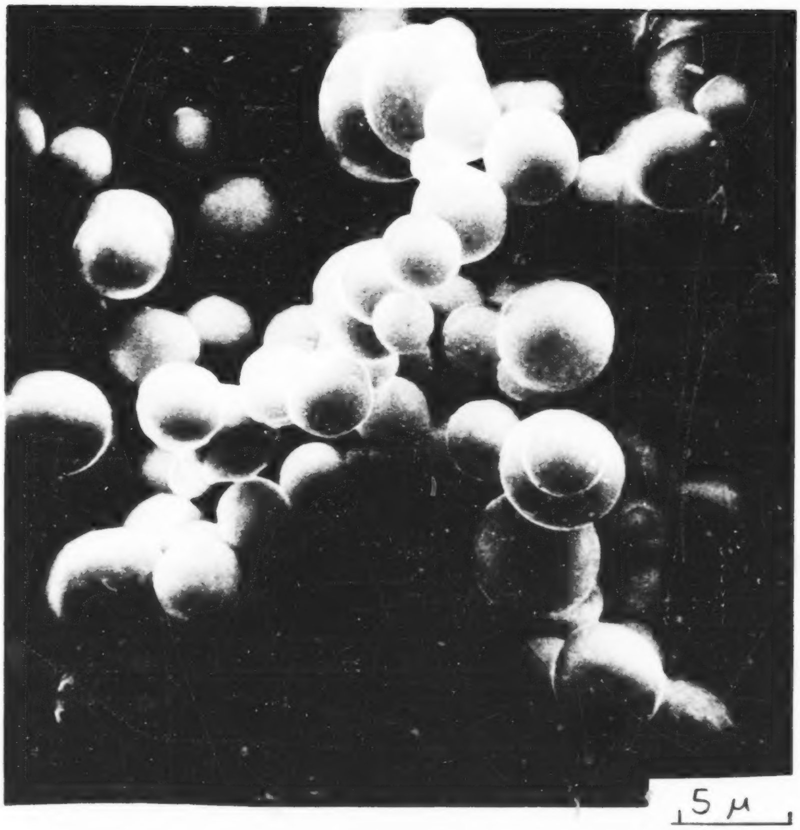

Figure 34. PbBrCl collected on quartz slide following the experimental scheme of Figure 25.

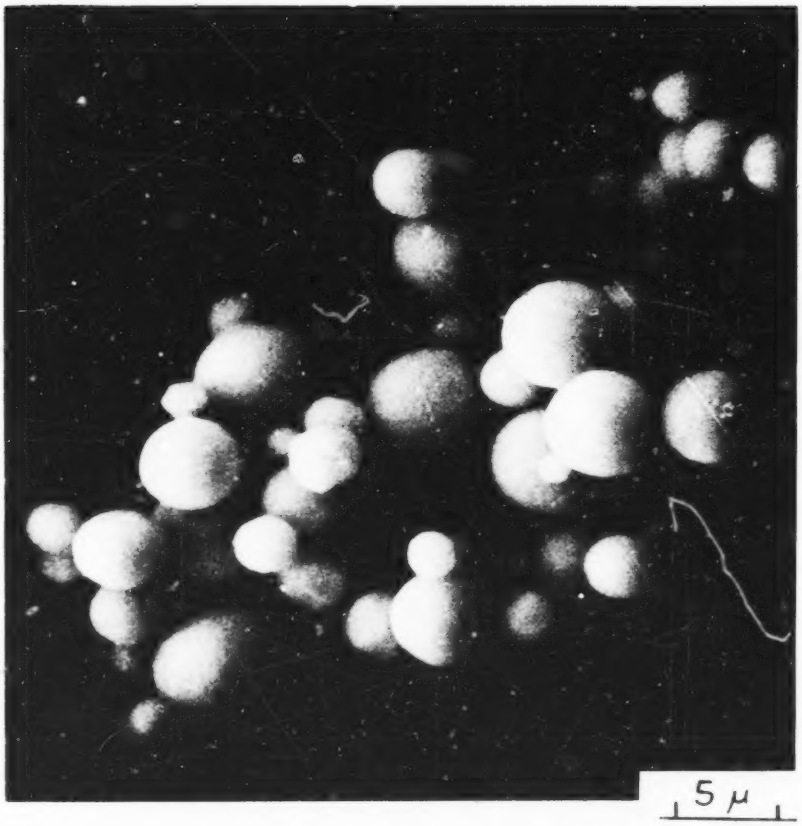

Figure 35. PbBrCl collected on quartz slide following the experimental scheme of Figure 25.

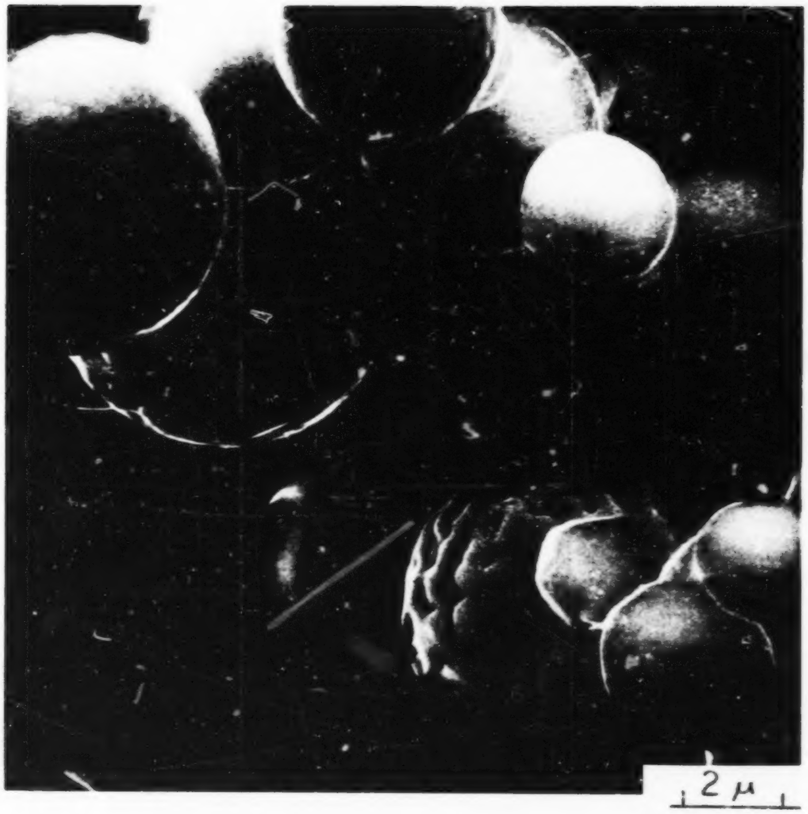

Figure 36. PbBrCl collected on quartz slide showing surface morphology.

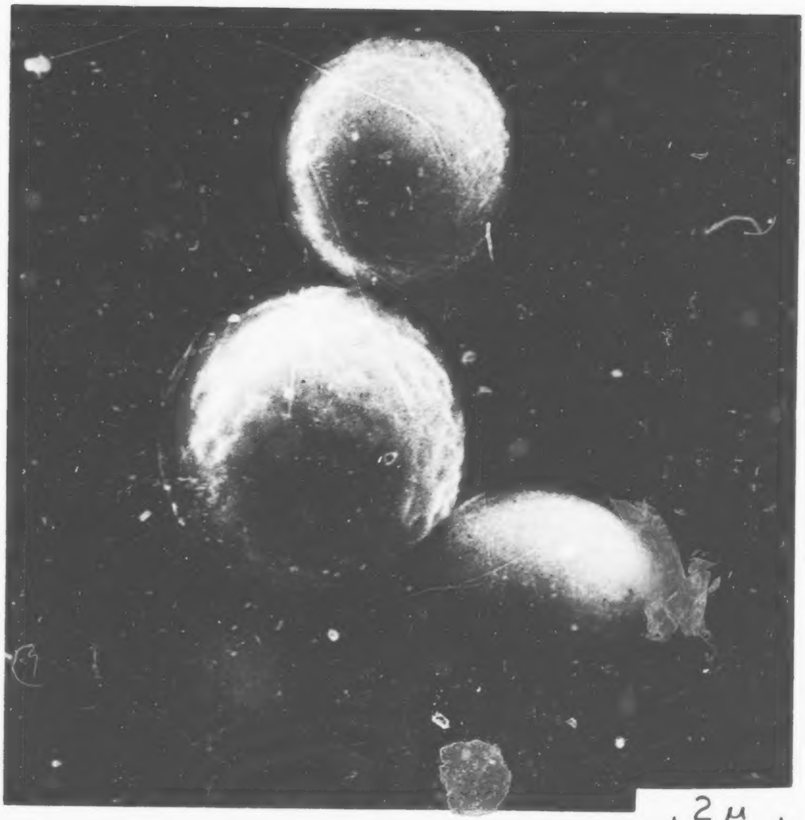

Figure 37. PbBrCl collected on quartz slide showing surface morphology.

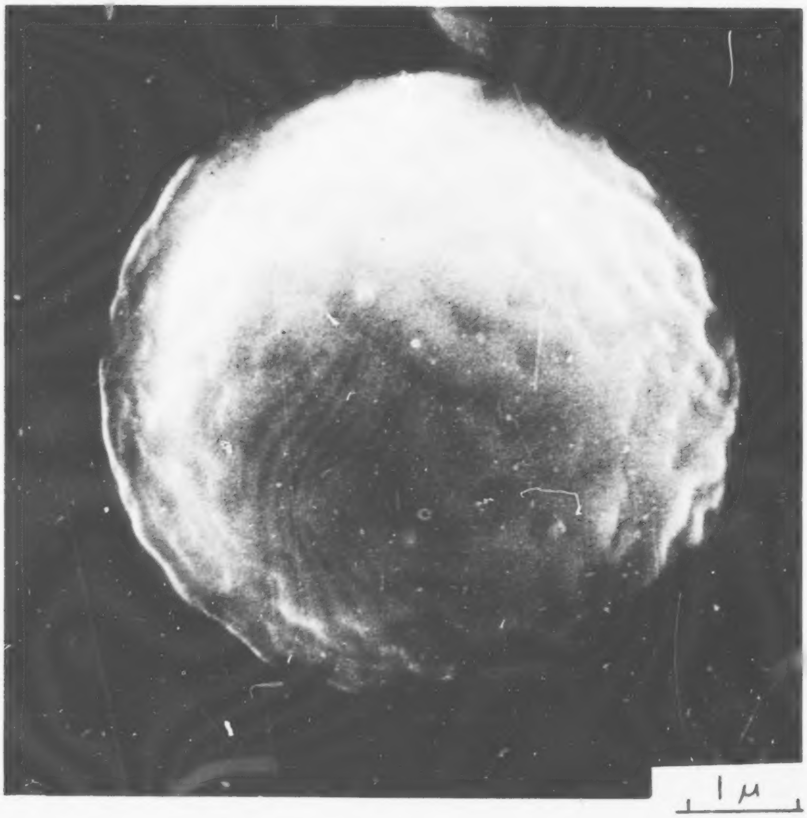

Figure 38. PbBrCl collected on quartz slide showing surface morphology.

charge characteristics of these particles will be investigated further.

Figure 39 is a scanning electron micrograph of a Reeve-Angel 934AH glass fiber filter. These filters were also used (in place of the quartz slide in Figure 25) to collect aerosol particles. Figure 40, a micrograph of PbBrCl particles collected on a fiberglass filter, clearly shows that particles smaller than the openings between the filter strands are collected. In fact the particles are apparently collected by electrostatic attraction to the filter strands and to other aerosol particles and not by being trapped between the filter stands. Figure 41 is an edgewise cut of the filter in Figure 40 Clearly, the great majority of the aerosol particles are trapped in the first 75 microns. Those few particles which appear deeper in the filter were probably displaced from the filter surface during the operation of cutting the filter. Because the average filter thickness was measured to be 640 microns (0.64 mm), these glass fiber filters are very effective for trapping lead halide aerosol particles.

b. Decomposition Studies Results. Table 21 lists the results of exposing laboratory pure lead halide aerosol particles to various conditions of ultraviolet radiation, humidity, carbon dioxide and temperature. As Table 21 shows, exposure of $PbBr_2$ and PbBrCl to high humidity, high CO_2 levels resulted in very little loss of halogen, especially compared to fresh automobile exhaust particulates, which have been observed to lose up to 75% of the bromine in the first twenty minutes after being exhausted (Robbins and Snitz, 1972).

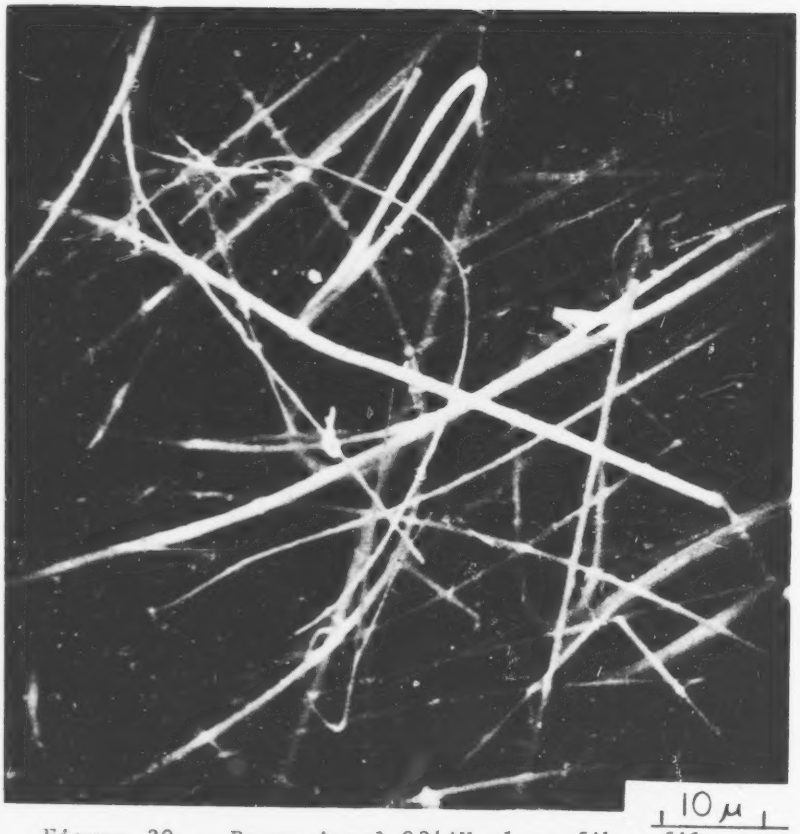

Figure 39. Reeve-Angel 934AH glass fiber filter.

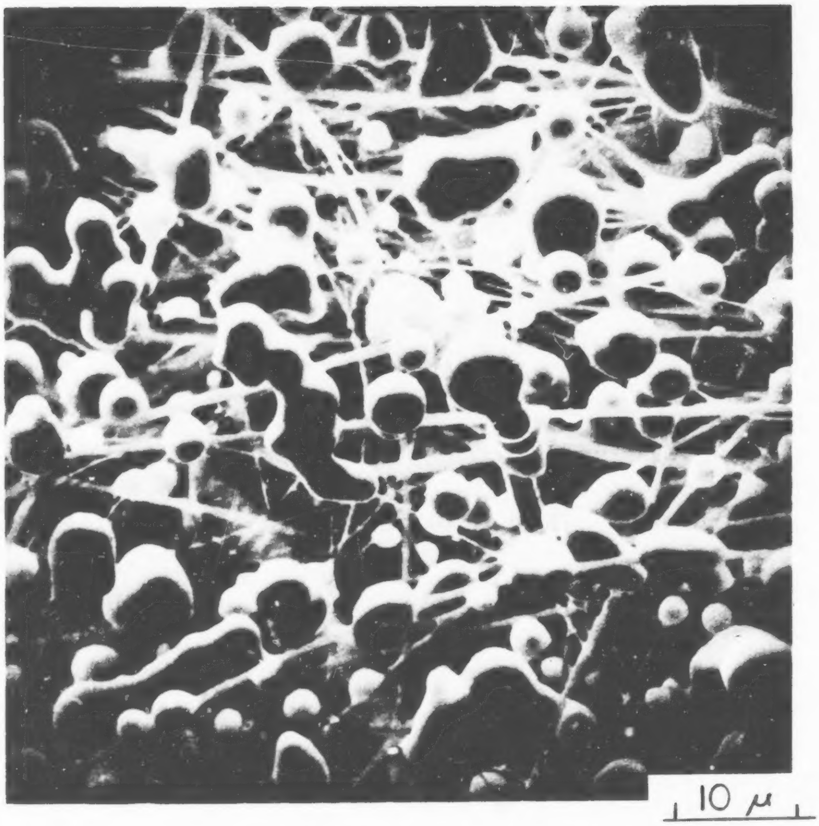

Figure 40. PbBrCl collected on glass fiber filter.

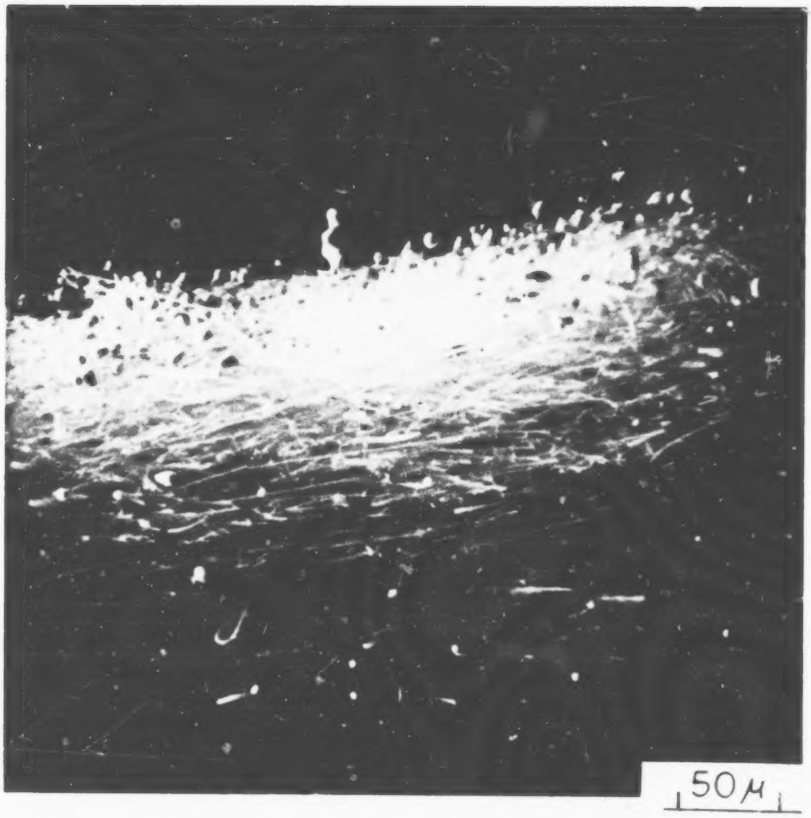

Figure 41. Edgewise cut of glass fiber filter with PbBrCl particles collected on upper face.

Table 21 and Figures 42 and 43 show that for laboratory pure lead halide aerosols, the only significant loss of halogen occurs as a result of irradiation by ultraviolet light. However, this loss is still small compared to the loss of halogen observed for automobile exhaust particulates.

B. The Movement of Lead in the Soil-Water System

1. Soil Accumulations of Lead

a. Background Lead and other heavy metal accumulations in roadside soils have been widely reported. Most of the data have been gathered from surface samples. Page (1971) reports on one site 76 m from the highway in which vertical distribution of lead is uniform through the depth of tillage, but declines immediately below. The difference in concentration between about 80 μg/g above and <1μg/g below indicates that leaching of lead is negligible. However, rainfall is low in the area near Los Angeles where the sample was collected.

b. Procedures We collected soil samples from a heavily travelled state route in our watershed area near Thomasboro, Illinois. The samples were collected as near the road as possible (1.5 m), at the bottom of a parallel drainage ditch (6 m), on the road side of the right-of-way fence (10 m), and 4 m into the tilled field outside the right-of-way fence. At each location, soil from 3 to 5 borings was combined according to increment of depth. The combined samples were oven dried, ground, and passed through a 40-mesh sieve prior to analysis. Subsamples (1 g) were extracted by shaking sequentially with three 15-ml portions of 1 N HCl (Lagerwerff and Specht, 1970). The soil-acid mixture was centrifuged and

Table 21· Decomposition of Lead Halides

Subjected to Various Conditions

	Sample and conditions	μeq halide left after decomposition	μeq halide lost during decomposition	Total μeq recovered halide	Theoretical μeq Pb and halide available	μeq Pb found by AA
1.	"pure" $PbBr_2$	-	-	69.2	68.4	65.8
2.	$PbBr_2$ 6.2 mWatt/cm^2 dry, CO_2 free air 32°C, 25 hr	(a)	7.94 (30.1%)	(a)	26.4	(a)
3.	$PbBr_2$ 9% CO_2, moist air 25°C, 24 hr	18.0	<0.05 (<0.3%)	18.0	18.0	18.4
4.	"pure" PbBrCl	-	-	292	294	297
5.	PbBrCl 9% CO_2, moist air 50°C, 72 hr	282	1.1 (0.4%)	283	288	288
6.	PbBrCl 6.2 mWatt/cm^2 dry N_2 32°C, 51.5 hr	176	9.6 (9.2 as Br_2) (0.4 as Cl_2) (5.1% of total)	186	190	194
7.	PbBrCl 6.2 mWatt/cm^2 dry, CO_2 free air 32°C, 50 hr	116	5.8 (5.4 as Br_2) (0.4 as Cl_2) (4.8% of total)	122	121	124

(a) not analyzed

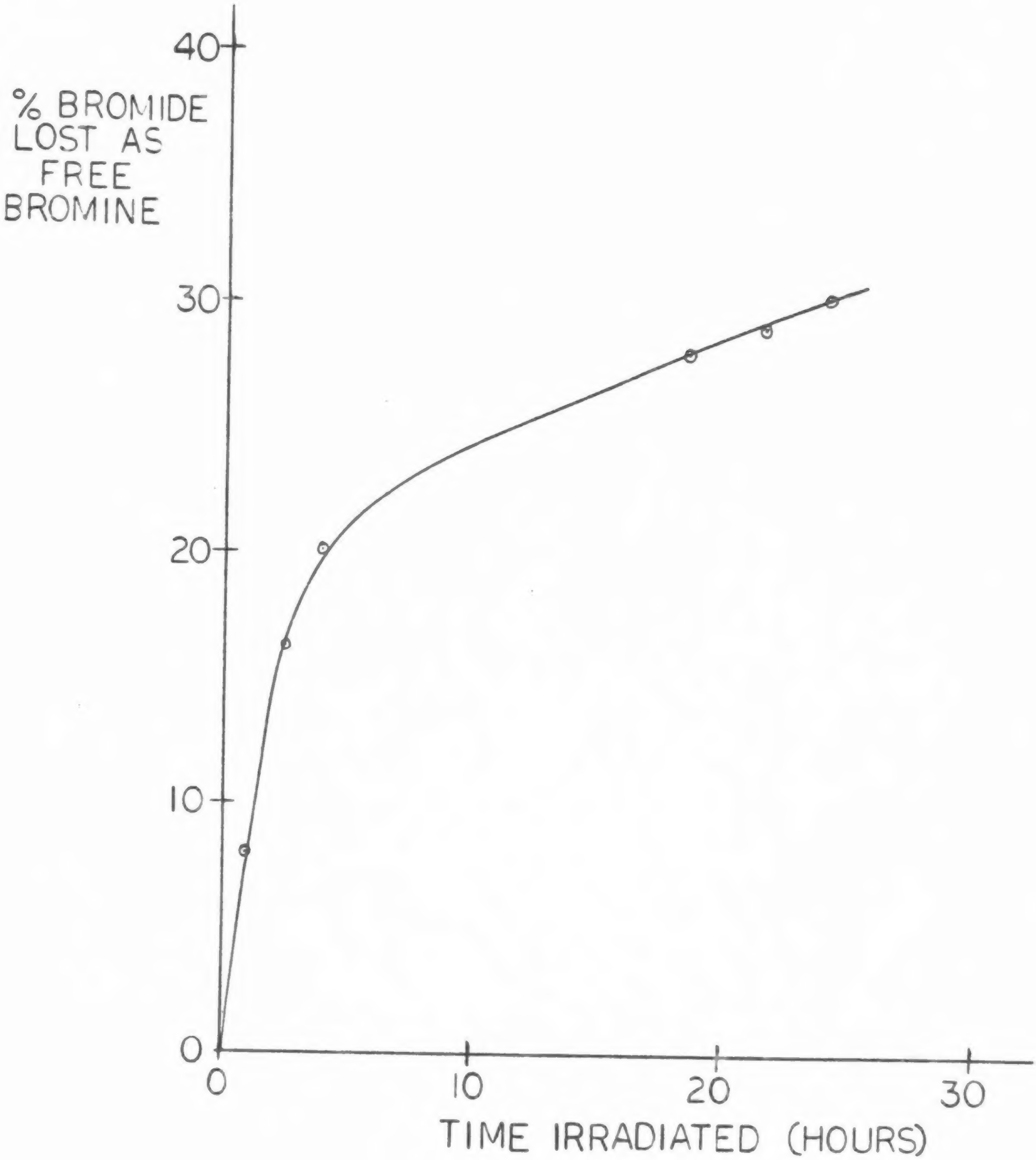

Figure 2. Photodecomposition of $PbBr_2$ in Air.

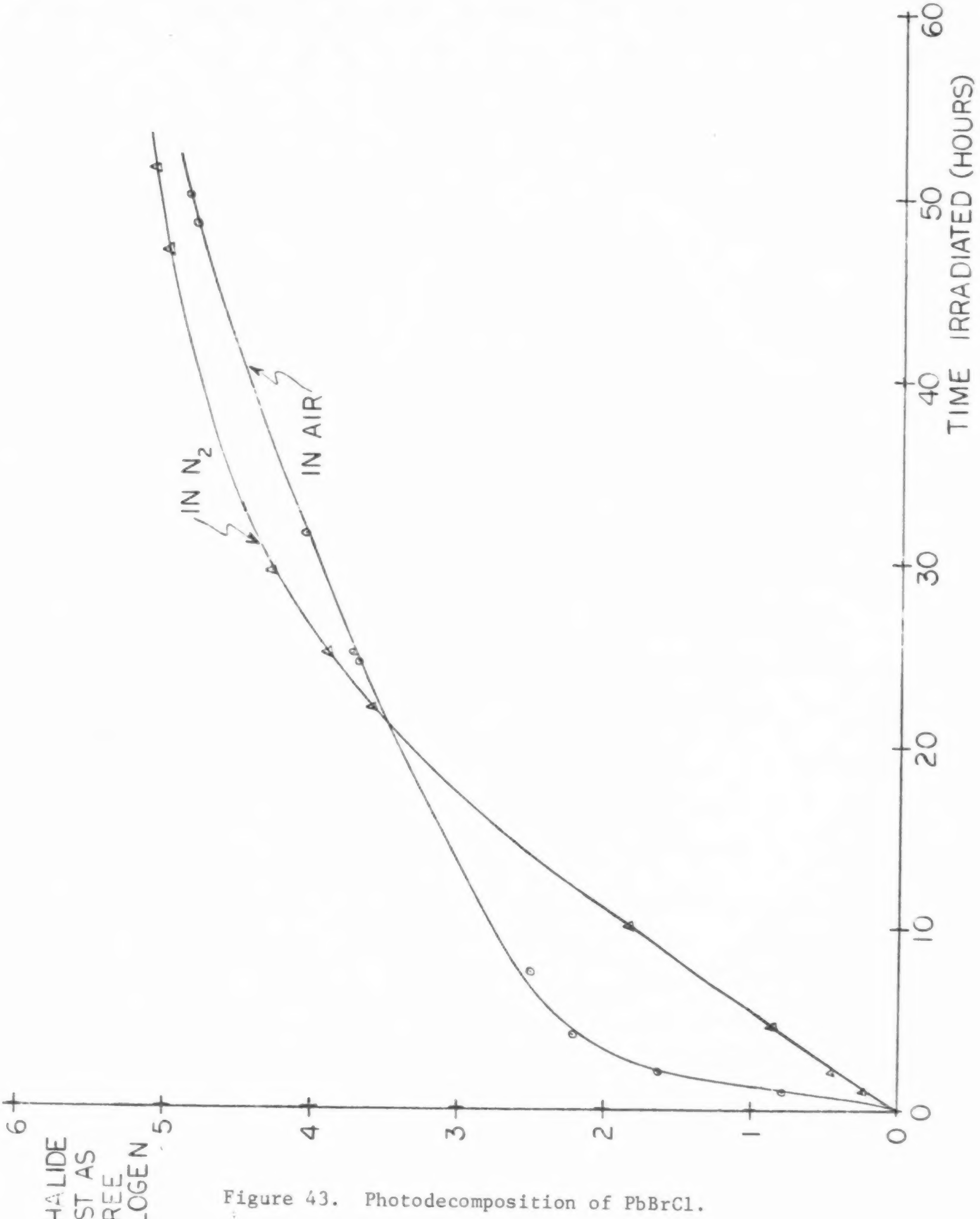

Figure 43. Photodecomposition of PbBrCl.

the supernatant solutions were combined and made to 50 ml volume. Lead was determined by atomic absorption spectrophotometry.

c. Results and Discussion Vertical distribution of lead in the soil profile is shown in Table 22. Values are means of two or more replicates. Lead concentration in surface soil samples decreased with distance from the roadside. In the roadside sample, which appeared to be composed of roadfill rather than native soil, somewhat elevated levels of lead were present to a 26 cm depth. The lead concentration in the surface layer was much higher (340 ppm) than in any other sample.

Only two depth samples were taken from the ditch. The surface contained less lead than the roadside sample, and the 26-35 cm layer contained the background level of lead.

The samples collected on the road side and the field side of the right-of-way boundary reflect the mixing of soil which takes place with tillage. The 0-7 cm layer of undisturbed soil contained 20 ppm more lead than did the cultivated soil. Neither soil showed lead deposition below 15 cm.

2. Mobility of Lead in Soils

a. Background Our data and that of Page (1971) indicate that there is little or no movement of lead downward in the soil profile. For our efforts to model the movement of lead within the defined ecosystem, a measure of the mobility of lead, if any, in the profile was needed. Several preliminary leaching experiments were carried out in order to assess the mobility of lead in soil.

Table 22 Lead concentrations with depth in soil sampled on the east side of Route 45 near Thomasboro, Illinois.

Location	Depth, cm	Pb, ppm
Roadside (1.5 to 2 m from pavement)	0-7	340.0
	8-15	47.0
	16-25	35.0
	26-35	15.6
Ditch (5 to 6 m from pavement)	0-7	65.0
	26-35	10.5
Right-of-way boundary (9 to 10 m from pavement, grass covered area)	0-7	44.0
	8-15	19.2
	16-25	16.5
	26-35	12.9
Field (10 to 14 m from pavement, 3 m inside plowed field)	0-7	24.0
	8-15	16.4
	16-25	12.6
	26-35	10.8

In addition to assessing the mobility of lead in soil, we also wished to evaluate methods for extraction of lead. In soil, lead may be incorporated in the crystal matrix of soil minerals, adsorbed on clay colloids, and/or complexed by insoluble soil organic colloids. Moreover, soluble lead-organic matter complexes and Pb^{++} cations may be present in soil solution. Thus the form of lead for which measurement is desired dictates the types of procedures which may be used for its analysis. The detection method, i.e., atomic absorption, colorimetry, anodic stripping voltammetry, can also limit the choice of procedure because of interferences from reagents included in a particular procedure. Soil samples amended with lead from various sources were analyzed as described below.

b. Procedures

1) Mobility Experiments Three soil columns 3.8 cm in diameter by 12.5 cm long were prepared with Flanagan silt loam A horizon and B horizon, and Plainfield sand A horizon. The top 2.5 cm of each soil was treated with a solution of lead acetate in an amount equal to 400 ppm lead oven dry soil weight. Filter paper was placed between the treated layer and the remainder of the column to allow a clean separation at the time of analysis. The soils were leached with 400 ml of a 100 ppm disodium EDTA solution. Leaching was carried out slowly over a 5-day period. After leaching, the soil columns were extruded and cut into 2.5 cm segments. Each segment was oven dried and ground. Duplicate subsamples (1 g) were extracted with 20 ml of refluxing concentrated HNO_3. The concentrated acid solutions was diluted appropriately for atomic absorption analysis.

A second leaching study included the use of a Soxhlet extractor and water. Two columns (4 cm in diameter x 5 cm long) of Flanagan silt loam surface soil were set up in sintered glass Soxhlet extraction cups. Field soil (2.5-cm layer) which had been amended with 3200 kg/ha lead as lead acetate was placed on one soil column (Baumhardt and Welch, 1972). The other was topped with a similar amount of soil amended at the time of addition with 1250 ppm lead as $PbBr_2$. The extraction cups were placed in a Soxhlet extractor with a vapor tube which bypassed the cup chamber, thus allowing operation at a temperature of about 50°C. Leaching was continuous for 14 days.

After leaching, the soil columns were separated into 2.5-cm sections. The soil was oven dried, mixed and 1-g subsamples were extraced with 1 N HCl.

In an additional experiment two soil columns (4 cm diameter x 7 cm long) of Flanagan silt loam having a cation exchange capacity (CEC) of 21 meq/100g were formed in Soxhlet extraction cups with sintered glass bottoms. These were placed in a Soxhlet extraction apparatus as previously described. Lead bromide and lead acetate in amounts calculated to saturate the entire soil column were added as undissolved salts on the soil surface. In this way, deposition of lead particulates was simulated.

The columns were leached with water continuously for 8 days. The soils were then removed and separated into 1-cm segments. Lead was extracted as previously described by shaking with 1 N HCl. Lead in the leachate comprised less than 1 per cent of that added.

2) Extraction Experiments Drummer silty clay loam was collected (0 to 15 cm depth) from field research plots amended with 50, 200, 1600 and 3200 kg/ha lead as lead acetate (Baumhardt and Welch, 1972). Saybrook silt loam was sampled at 0 to 7.5, 15 to 20, and 30 to 33 cm

depths in field lysimeters which had been irrigated with liquid digested sewage sludge during the growing seasons of the three previous years. Totals of 68.6 and 34.3 cm of digested sludge had been added. The digested sludge was obtained from the Calumet treatment plant of the Metropolitan Sanitary District of Greater Chicago and was of mixed domestic and industrial origin. Roadside soil was sampled (0 to 7.5 cm depth) at distances of 2 and 10 m from a highway on the east side near a busy intersection in Champaign, Illinois. All soils were oven dried, ground, and passed through a 40-mesh sieve prior to analysis. Lead was detected with atomic absorption spectrophotometry. All data reported are based on analyses of two or more replicate samples.

For concentrated HNO_3 extraction, a procedure similar to that of Singer and Hanson (1969) was used. Soil (1.0 g) and 20 ml of concentrated HNO_3 were refluxed 4 to 6 hours, but no addition of sulfuric or perchloric acids was made. The final volume, 25 to 100 ml, was chosen on the basis of expected lead concentration in the soil.

The 1 N HCl extraction was carried out according to a procedure reported by Lagerwerff and Specht (1970) which was modified as follows. Soil samples (1.00 g) were sequentially extracted by shaking (15 min.) with two 10-ml portions of acid. Solids were settled by centrifugation and the supernatant solution was made to 50 ml or appropriate volume.

One-gram soil samples were ashed at 500°C, a temperature which should not allow significant volatilization of lead (Gorsuch, 1959). Following 15 to 18 hours of ashing, 5 to 8 ml of 6 N HCl was added to the ash and heated to boiling. The acid-residue mixture was then made to volume with distilled water.

Soil samples (1.00 g) were extracted with a solution containing 0.01 M EDTA and 1.0 M ammonium carbonate at pH 8.6 (Trierweiler and Lindsay, 1969). The procedure was the same as for 1 N HCl extraction above.

c. Results and Discussion

1) Mobility Experiments Distribution of lead with depth in the soils is shown in Figure 44. Some lead was leached into the 5-cm layer, but no detectable amounts were found below it. Extraction recoverability in the treated layer appeared best for the Plainfield sand. Lack of organic matter and clay colloids probably allowed for more complete release of lead. EDTA was chosen because it has a strong affinity for lead. Where large amounts of lead were leached downward or removed from the soil column, it could have been predicted that solutions less reactive with lead might displace it. Since little lead was moved with EDTA, the strong affinity of lead for the inorganic and organic soil colloids was demonstrated.

Leaching losses were small. Lead which was leached from the soils probably came from the native supply in the lower portion of the soil column. Flanagan A horizon yielded 8 μg, B horizon 6.8 mg, and Plainfield sand 8.5 mg of lead.

Lead distribution through the soil column after Soxhlet extraction is shown is Figure 45. Neither soil showed significant changes in lead concentration below the spiked top layer. This indicated that lead was held strongly enough by soil colloids that a displacing ion or chelating agent would be required in the leaching solution in order to mobilize the lead.

When cation exchange sites are saturated with a given cation, movement of that cation through soil should be facilitated. To test this hypothesis, an experiment incorporating soil columns was designed.

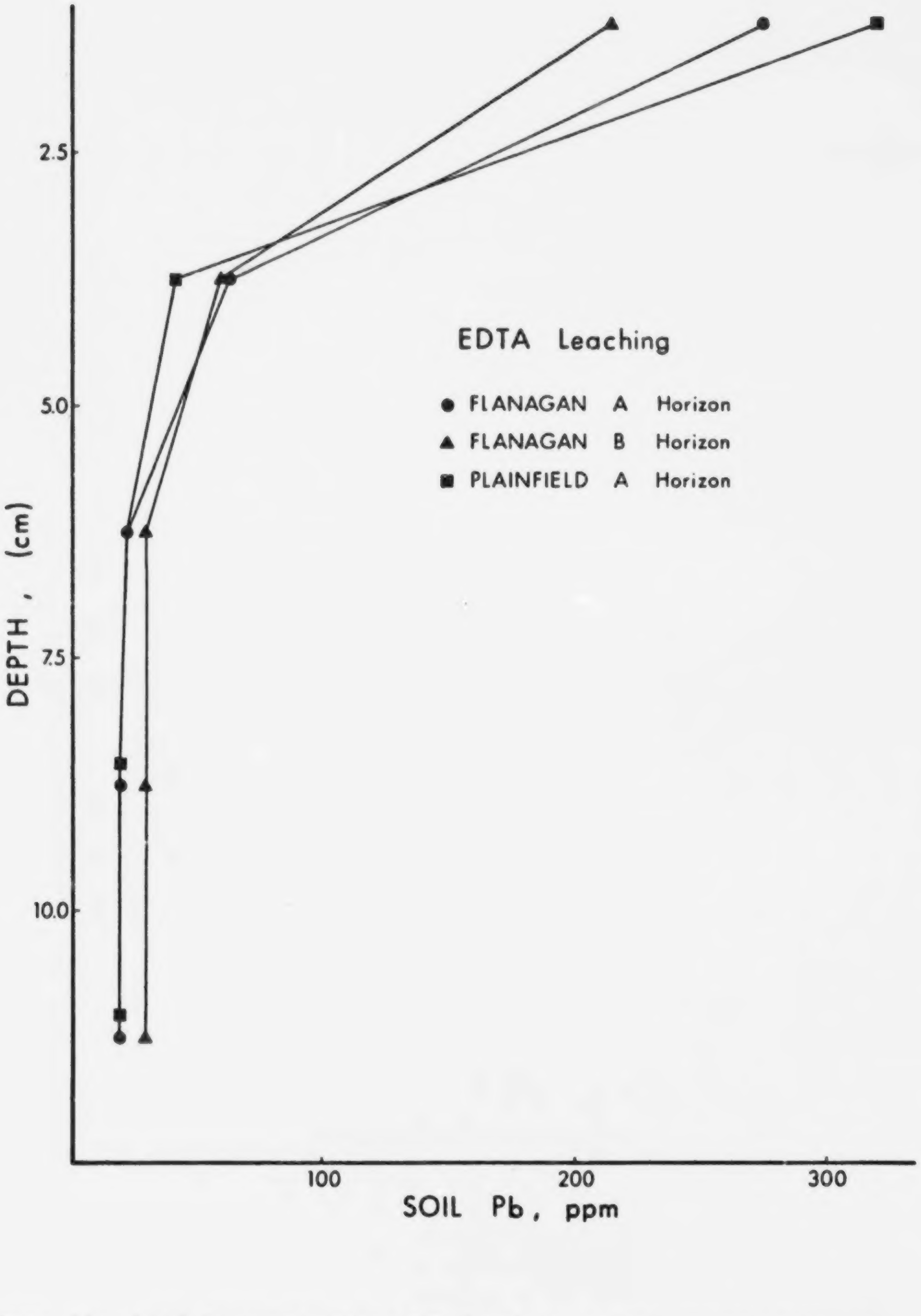

Figure 44. Lead Concentration with Depth in a Soil Column Following Leaching with Disodium EDTA.

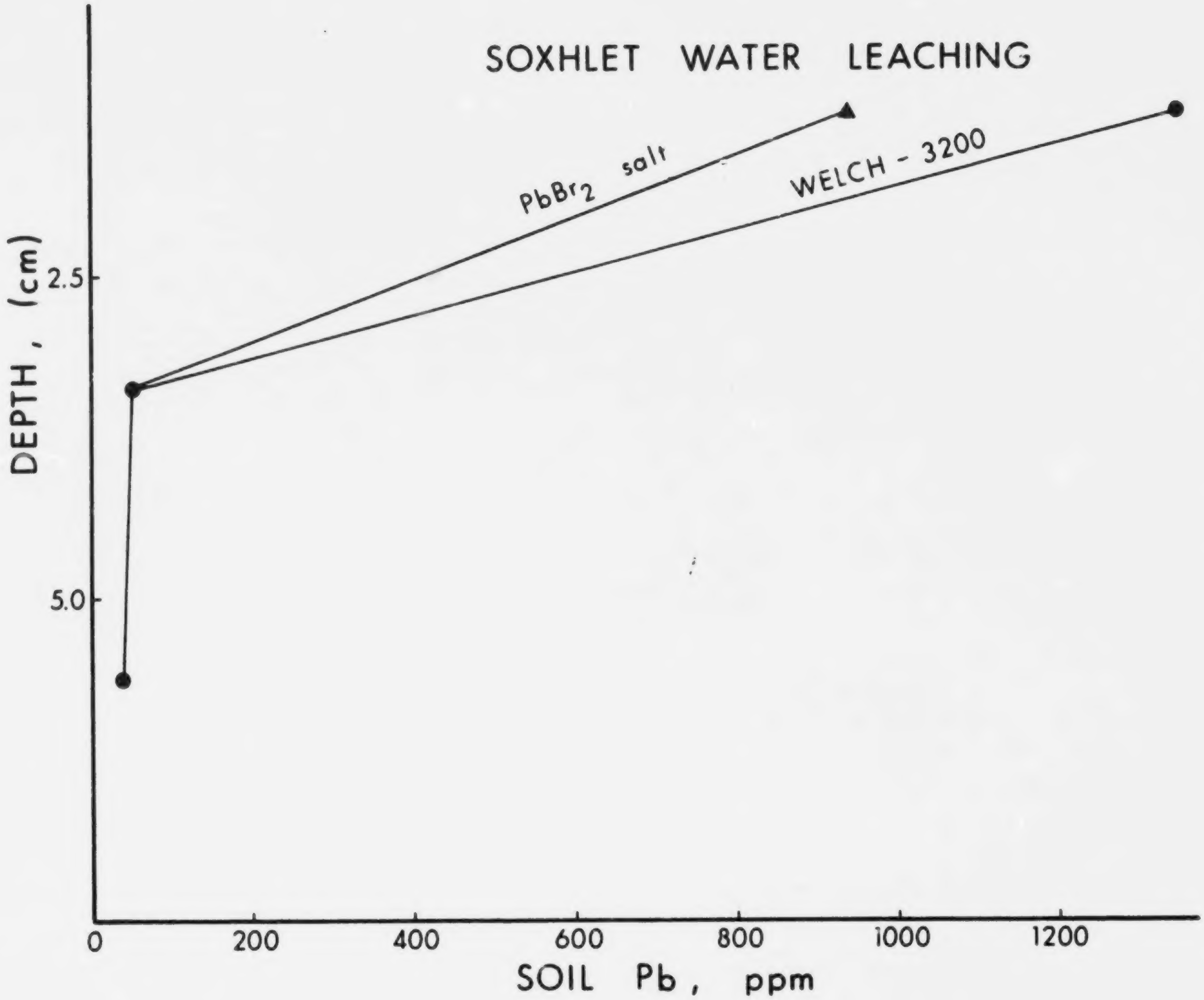

Figure 45. Lead distribution in a soil column following 2 weeks of continuous leaching with water.

A plot of lead distribution in the soil columns is shown in Figure 46. The curve for lead acetate shows a distribution which was predicted. The $PbBr_2$ distribution, expected to be similar to the acetate, was not. Soil from 1-6 cm in the lead acetate-treated column contained about the same lead concentration. A saturation of absorption sites by lead would produce such a distribtuion. The higher initial concentration could be due to chemical precipitation of some lead. The lower concentration in the lowest soil increment could have resulted from the failure of the lead accumulated in the top layer from being adsorbed below.

Lead bromide was expected to behave similarly because Pb^{++} cation and not the salt form should be the active species. Lead bromide is less soluble and required several hours to disappear from the soil surface. Solubility may have played a role, but no ready explanation exists for the declining lead concentration with depth.

2) Extraction Experiments. A few characteristics of soils which were utilized in the extraction study are listed in Table 23. the pH of Drummer soil was a unit lower than is usual for that soil type, whereas the pH of Saybrook was typical. High organic carbon concentrations in sludge-amended soil reflect organic residue from the sludge. Sludge accumulation at the maximum application rate represented about 200 metric tons dry solids per hectare. Organic carbon was not chemically determined in the roadside soil, but was estimated to be less than 1 per cent because the soil appeared to be subsoil fill.

Lead determinations were made with dry ashing and several extraction procedures. Destruction of organic matter coupled with strong acid extraction of residue as in the dry-ashing method should effect quantitative

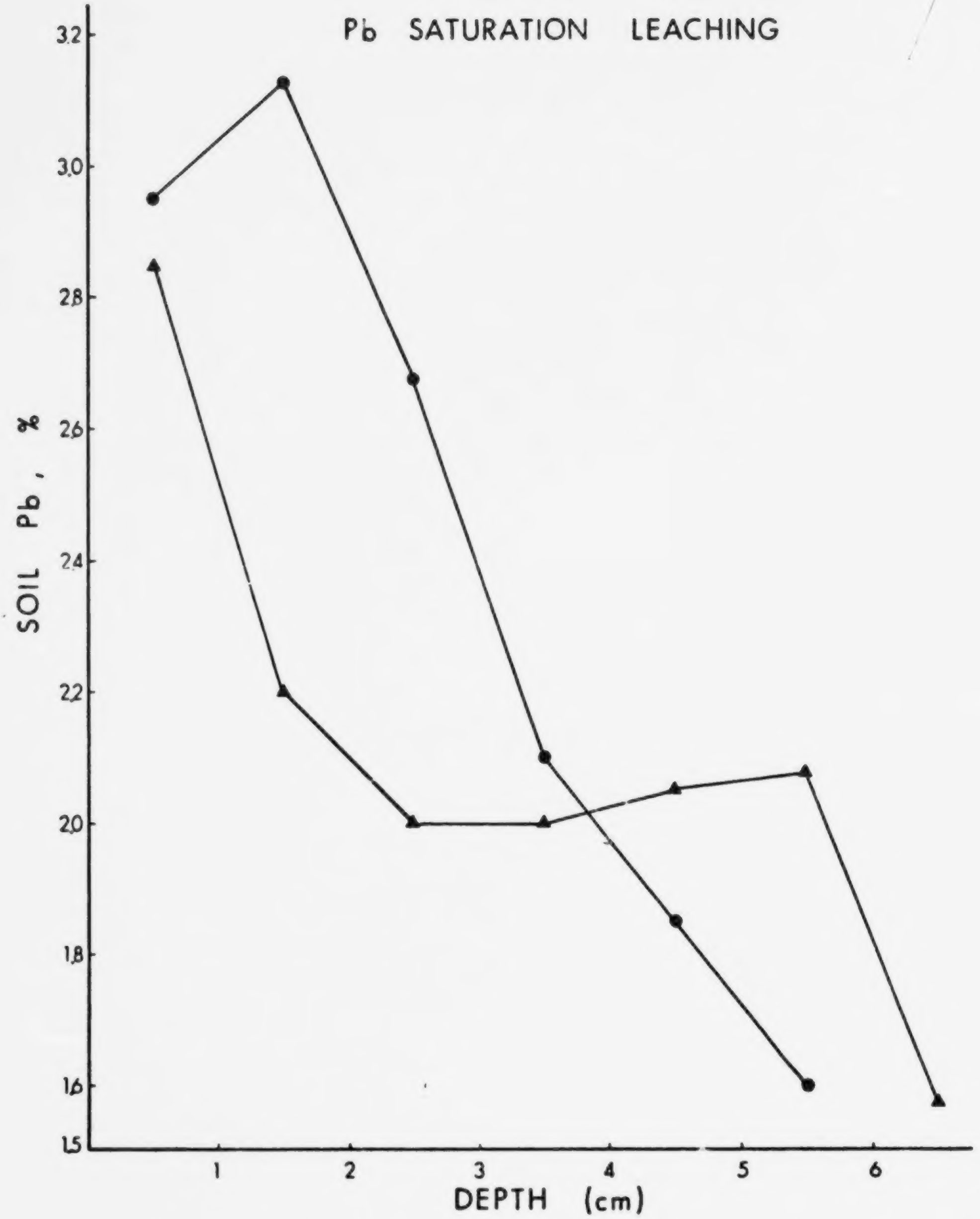

Figure 46. Lead Distribution in Soil Following Addition of Pb Acetate (Triangle) and $PbBr_2$ (Circle) in Amounts Calculated to Saturate the Adsorption Sites in Soil.

Table 23.

Characteristics of Surface Soils.

Soil type	Treatment	pH	CEC meq/100g	Organic Carbon %
Drummer silty clay loam	All	5.9	34.0	4.80
Saybrook silt loam	68.6 cm sludge	6.1	35.5	8.76
	34.3 cm sludge	6.1	39.8	5.71
	0 cm sludge	5.5	33.8	3.12
Roadside fill	--	6.3	7.5	--

recovery of lead. However, lead and lead halides in particular are volatile at temperatures over 500°C. Gorsuch (1959) recovered 97 to 100% of the lead in plant tissue ashed at 450°C, but only 71 to 83% at a 650°C ashing temperature. With pure $PbCl_2$, only 20 to 25% of the lead could be accounted for after ashing at 650°C. He concluded that lead recovery was quantitative up to a temperature of 500°C.

Singer and Hanson (1969) reported 93 to 95% recovery of lead with refluxing concentrated HNO_3. Concentrated HNO_3 should oxidize most of the organic matter and release lead from adsorption sites on clay colloids. Results below show that this method was not always as effective as others tested.

Lagerwerff and Specht (1970) did not report lead recovery with 1 N HCl, but stated that complex formation of Pb (II) with the acid reaches a maximum at 1 N concentration. With soil amended with $PbBr_2$, we observed 96 to 104% recovery with the 1 N HCl method described above.

The EDTA-ammonium carbonate (EDTA-AC) extraction procedure was published as a method for determining "plant available" Zn (Trierweiler and Lindsay, 1969). Another phase of the lead research was concerned with measuring lead which is "available" to plants. The EDTA-AC extraction was tried and found to be more quantitative than indicative of the "available" lead fraction. The stability constant of the Pb-EDTA complex ($\log K = 18.2$) is greater than the Zn-EDTA complex ($\log K = 16.3$) which accounts at least in part for the difference in the thoroughness of extraction.

Lead concentrations in Drummer soil are given in Table 24. There were no significant differences between extractants at the 50 and 200 kg/ha lead treatment levels. Although replicates agreed, the 175 ppm value for 1 N HCl extraction of soil from the 200 kg/ha treatment was probably spurious. At higher lead treatment levels, dry ashing and concentrated HNO_3

Table 24.

Mean values of Pb concentration in Drummer silty clay loam as determined with several procedures (ppm oven-dry soil weight).

Analytical procedure	Pb treatment, kg/ha			
	50	200	1600	3200
Dry ashing 500°C	53	96	502	950
Concentrated HNO_3 extn.	44	50	462	805
1 N HCl extn.	65	175	640	1244
EDTA-AC extn.	36	64	625	1190

LSD (0.05) = 78

Table 25.

Lead concentration means in Saybrook silt loam as determined with several procedures (ppm oven-dry soil weight).

Analytical procedure	Digested sludge accumulation, cm		
	68.6	34.3	0
Dry ashing 500°C	256	158	28
Concentrated HNO_3 extn.	208	103	24
1 N HCl extn.	267	154	20
EDTA-AC extn.	152	110	22

LSD (0.05) = 43

extraction were similar as were 1 N HCl and EDTA-AC extractions. Based on the arbitrary value of 2 million pounds as the weight of soil in an acre plow layer, a concentration of 1425 ppm lead in soil at the maximum rate was calculated.

Lead is a ubiquitous component of municipal sewage treatment plant sludges. Industrial effluents may influence its concentration, but lead is also present in sludge from domestic sources. Since sludge disposal on land is a practice which is gaining in popularity, it introduces another means of lead dispersal to the environment. Therefore, we included sludge-amended soil in this study.

Table 25 lists lead concentrations in Saybrook soil as a function of sludge treatment and analytical method. With this soil, dry ashing and 1 N HCl extraction are not significantly different at any treatment level. At the 2 lower treatment rates, EDTA-AC and concentrated HNO_3 extractions are not significantly different. However, each pair of procedures was significantly different at all but the 0-sludge treatment. EDTA-AC extraction gave particularly low results with the soil receiving maximum sludge treatment. This results from the fact that heavy metals occur primarily as chemical precipitates in the solid phase of sludge (Jenkins and Cooper, 1964). Acidic extraction removes metals precipitated as sulfides and hydroxides as well as those adsorbed. A chelating agent at alkaline pH would not be expected to react as effectively with metal precipitates.

Data reported by Geering and Hodgson (1969) and others indicate that movement of heavy metals in soil solution occurs primarily through formation of organometallic chelates. The soil which had the highest potential for chelate formation because of organic matter content was sludge-amended Saybrook. It was sampled below the zone of cultivation and extracted with

1 N HCl. Lead concentrations with depth are presented in Table 26. There is no evidence that lead had leached significantly during the 3 years of sludge application and weathering. Leaching studies in the laboratory also confirm the immobility of lead. It is unlikely that leaching of lead through a soil profile would be great enough to pose a threat to tile drain or ground water quality.

Soil samples were collected from the downwind side of a busy street where vehicles generally accelerate. That lead from combustion of tetraethyl lead accumulates in roadside soil is well established (Cannon and Bowles, 1962; Chow, 1970; Lagerwerff and Specht, 1970; Motto, Daines, Chilkoe, and Motto, 1970; Page, Ganje and Joshi, 1971; Singer and Hanson, 1969). Four soil samples were composited from 2 m and 10 m from the road. Table 27 lists lead concentrations as a function of distance and analytical procedure. Lead concentration was lower in soil 10 m from the road than in soil 2 m distant. EDTA-AC and 1 N HCl extractions, and EDTA-AC and concentrated HNO_3 and EDTA-AC extractions were not significantly different. Dry ashing removed significantly more lead from the 2-m sample than other methods, and almost the same amount as 1 N HCl from the 10-m sample.

We are currently determining the lead remaining in residues of these soils after extractions with the above methods (John, 1971). In this way, the total lead content can be determined and the efficacy of the method compared.

3. Cation Exchange Studies

a. Background. Clays and organic matter in the soil have very high capacity to hold cations and certain anions and they serve as reservoir

Table 26.

Means of 1 N HCl extractable Pb with depth in Saybrook silt loam irrigated with liquid digested sewage sludge (ppm oven-dry soil weight).

Depth in cm	Digested sludge accumulation, cm		
	68.6	34.3	0
0 - 7.5	267	158	18
15 - 20	19	20	20
30 - 33	18	14	15

Table 27.

Lead concentration means for roadside soil from two locations near a highway (ppm oven-dry soil weight).

Analytical method	Distance from highway, m	
	2	10
Dry ashing 500°C	330	262
Concentrated HNO_3 extn.	234	172
1 N HCl extn.	283	267
EDTA-AC extn.	252	211

LSD (0.05) = 40

of nutrients for plant growth (Broeshart and Fried, 1967). Because of the extreme importance of this property of the clays, they have been studied in great detail, especially in regard to their ion exchange properties. Among the clay minerals, montmorillonites and vermiculites were found to have the highest cation exchange capacity (∿ 100 me/100g) (Grim, 1968).

In our studies related to plant uptake of lead from soil and solutions, we were interested in the ion exchange equilibria between the clays and lead in solution. Overstreet and Krishnamurthy (1950) used the equilibrium reaction of calcium saturated clay with Pb^{++} and also other reactions to test the validity of their ion exchange equations derived from statistical thermodynamics. Mackenzie (1963) reported that the K^+, Rb^+, Sr^+ and Pb^{++} on montmorillonites (smectites) was rendered nonexchangeable by drying the clay. Besides these two reports, no information is available on the lead ion exchange behavior of clays and vermiculite.

b. Procedures.

1) Clays. Montmorillonite, because of its high cation exchange capacity (CEC) was selected for our clay-Pb^{++} studies. It was obtained from Wyoming through Wards Natural Science Establishment Inc., Rochester, New York. A fresh sample of montmorillonite was washed several times with deionized water to remove any soluble matter held to it. Particles of size less than 2 μ were then separated by sedimentation techniques and used throughout this work. Specific cation saturated montmorillonite was made by equilibrating clay for 24 hours with 1 M acetate solution of the cation of interest. The acetate solution was replaced

twice during the equilibration to ensure the saturation with the desired cation. The clay was then separated by centrifuging and the washing procedure, dispersing in deionized water and centrifuging, continued until the clear liquid above the clay showed a specific resistance of greater than 10^6 ohms.

The montmorillionite was always stored as a dispersion in doubly-distilled water and known aliquots of this uniform dispersion were used in our studies. The CEC of the clay was determined by replacing the K^+ from potassium-saturated clay with 0.5 M calcium acetate and determining the K^+ released by atomic absorption spectrometry.

In the equilibrium studies of potassium-saturated montmorillonite with Pb^{++}, the following procedure was used. Different solutions of 100 ml volume were made to contain fixed amounts of KNO_3, potassium-saturated clay and varied amounts of lead nitrate. The solutions were shaken every 1/2 hour during the first 6 hours of the total equilibration time of 24 hours and then centrifuged to separate the solution from the clay. The lead concentration in the clear liquid was determined polarographically after transferring it into a cell and making it 20 mM in KNO_3.

2) Vermiculite. Hoagland's or water solutions were amended with 10 or 100 ppm Pb and 530 ml added to 100 g of vermiculite. After a two-day equilibration period, the excess liquid (60-100 ml) was drained off and sent to the analytical laboratory for lead analysis.

c. Results and Discussion.

1) Clays. Polarographic determinations of the amount of Pb^{++} exchanged were calculated from the amount of Pb^{++} added initially

and the amount found in solution at equilibrium. The results obtained over a 100-fold change in concentration of Pb^{++} (at equilibrium) are presented in Table 28.

At equilibrium lead concentrations >1.84 mM, the values of lead exchanged/g of montmorillonite (last column, Table 28) are spread around the CEC of montmorillonite, 0.885 me/g. Evidentaly the saturation of montmorillonite with Pb^{++} was achieved even when the K^{+} concentration in solution is in 50 fold excess over Pb^{++} at equilibrium. However, at equilibrium lead concentrations >1.84 mM, the value of lead exchanged increased beyond that expected from the CEC of montmorillonite. This excess lead held by montmorillonite over its CEC is apparently due to some process other than cation exchange.

The results of the experiments similar to the above carried out with calcium saturated montmorillonite are shown in Table 29. The values in the 1st column of Table 29, show that the lead ion exchange increased with equilibrium Pb^{++} concentration in solution but levelled off at about 0.66 me/g, a value about 25% less than the CEC value. It appears as if some of the Ca^{++} on montmorillonite is not replaceable under the conditions of the experiment.

The ease of exchangeability of a cation on clays, has been shown by Jenny and Ayers (1939) and also by Wiklander (1946) to be a factor of the degree of saturation of the clay with that ion and also of other cations occupying the remainder of the exchange sites. Wiklander (1946) reported that Ca^{++} became more and more difficult to be exchanged as its concentration on the clay is decreased. The data in Table 29 are indicative that the last 25% of the Ca^{++} in calcium-saturated clay is not easily exchangeable by Pb^{++}. From their studies with monovalent and polyvalent cations on

Table 28. Equilibrium Studies of K^+ Saturated Montmorillonite with Pb^{++} in Solution

Weight of Montmorillonite (MM)	40.3 mg
Volume of solution	100 ml

Initial Concentrations mM		Equilibrium Concentration mM	Pb^{++} exchanged me/g of MM
K^+	Pb^{++}	Pb^{++}	
2.00	0.20	0.042	0.785
2.00	0.50	0.322	0.835
2.00	1.00	0.82	0.89
2.00	1.50	1.33	0.84
2.00	2.00	1.89	0.80
2.00	2.50	2.22	1.4
2.00	3.00	2.75	1.2
2.00	3.50	3.28	1.1
2.00	4.00	3.76	1.2

Cation exchange capacity determined from the K^+ released by Ca^{++} from K^+ saturated montmorillonite 0.885 me/g

Table 29. Equilibrium Studies of Ca^{++} Saturated Montmorillonite with Pb^{++} in Solution

Weight of Montmorillonite	54.3 mg
Volume of Solution	100 ml

Initial Concentrations mM		Equilibrium Concentration mM	Pb^{++} exchanged me/g of MM
Ca^{++}	Pb^{++}	Pb^{++}	
2.0	0.2	0.162	0.140
2.0	0.5	0.417	0.306
2.0	1.0	0.864	0.501
2.0	1.5	1.32	0.66
2.0	2.0	1.79	0.77
2.0	2.5	2.32	0.66
2.0	3.0	2.93	0.63
2.0	3.5	3.32	0.66
2.0	4.0	3.82	0.66

Cation exchange capacity determined from the K^{+} released by Ca^{++} from K^{+} saturated montmorillonite 0.885 mg/g clays, Mering and Glaeser (1954) found that montmorillonite with 30% of its CEC satified by Ca^{++} and 70% by Na^{+}, behaved like a sodium saturated clay. From the above discussion, it is clear that the exchange behavior of Ca^{++} on montmorillonite varies with its concentration on the clay. Marshall (1964) discussed these changes in terms of changes in bond energy of the cation with the clay.

The first three sets of data in Table 29 were obtained under conditions similar to those used by Overstreet and Krishnamurthy (1950) and thus a comparison is possible. The exchange of Ca^{++} on Utah bentonite with Pb^{++} was reported to follow the simple mass action equilibrium

$$K = \frac{[Pb^{++}\text{ clay}]\ [Ca^{++}]}{[Ca^{++}\text{ clay}]\ [Pb^{++}]}$$

with an equilibrium contant, K, of 1.40. Similar calculations with our data, show that mass action equilibrium is valid here also, leading to a K value of 1.45. However, when the Ca^{++} concentration on the clay (montmorillonite) fell below 25% of its saturation level, the simple mass action equilibrium expression was found to be invalid.

The procedure described above for making a specific cation saturated montmorillonite was not found to be very successful with Pb^{++}. The problem arose in washing off the excess Pb^{++} held on to clay after the saturation process. Even after the eighth wash of the clay with deionized water, the concentration of Pb^{++} was still present in the wash solution at a concentration of the order of 5×10^{-4}M. Furthermore, the concentration of Pb^{++} in the 6th, 7th, and 8th wash solutions showed no significant change in concentration. It appears as if some lead acetate was held in or adsorbed on the clay and released slowly into the solution. More work is needed, to find out exactly what is causing this seemingly excess uptake of Pb^{++}

and its slow release into water on equilibration. It is planned to substitute lead acetate by other lead salts like lead nitrate and study how the clay behaves with these salts.

2) Vermiculite. When a lead-amended solution was added to vermiculite, virtually all of the lead was adsorbed on the vermiculite and was not in solution (Table 30). Because of this adsorption where a constant low concentration of lead is required in the growth nutrient media, it is suggested that vermiculite be eliminated from consideration as a substrate. Instead, we have chosen to use a completely hydroponic growth medium for the uptake studies to be reported later in this section.

Table 30.

Adsorption of Lead by Vermiculite.

Treatment Solution	Theoretical Pb concentration (ppm)	Pb concentration determined by AA (ppm)	Pb concentration in vermiculite solution (AA analysis)(ppm)
Distilled H_2O	10	10	<1.1
Distilled H_2O	100	99	<1.1
+P Hoagland's solution	10	4.5	<1.1
-P Hoagland's solution	10	11	<1.1

C. Movement of Lead (Cadmium) in the Soil-Water-Organismal System

1. Adsorption and Exchange of Lead on Root Surfaces

a. Background There are two distinct mechanisms by which ions or salts become associated with plant roots (Kramer, 1969). First, the roots have certain cation exchange capacity (CEC) like in clays and synthetic resins and they exchange cations in a rapid, reversible and non-selective manner. Second, the ions are transported into the roots by a metabolic process which is very slow, irreversible and highly selective to both cations and anions. Both of these processes take place simultaneously when plant roots in distilled water are transferred into a salt solution. Since the cation exchange by the roots is limited by the CEC, it ceases after a short time while the absorption by metabolic process continues. Epstein and Leggett (1954) and also Fried et al. (1958) clearly demonstrated the time dependence of exchange and metabolic absorption at barley roots using radiotracers. Since it is important to distinguish between these two processes in our studies of cation uptake by roots (and plants in general), studies were made with corn plant roots and lead ions and they are reported below.

b. Procedures

1) Root Wash Experiments Roots of lead treated sunflower and corn were washed in deionized water, 5mM $caCl_2$, or 20 mM EDTA. The washes were serial and each lasted for one hour. Further experimental details are presented in Figure 47.

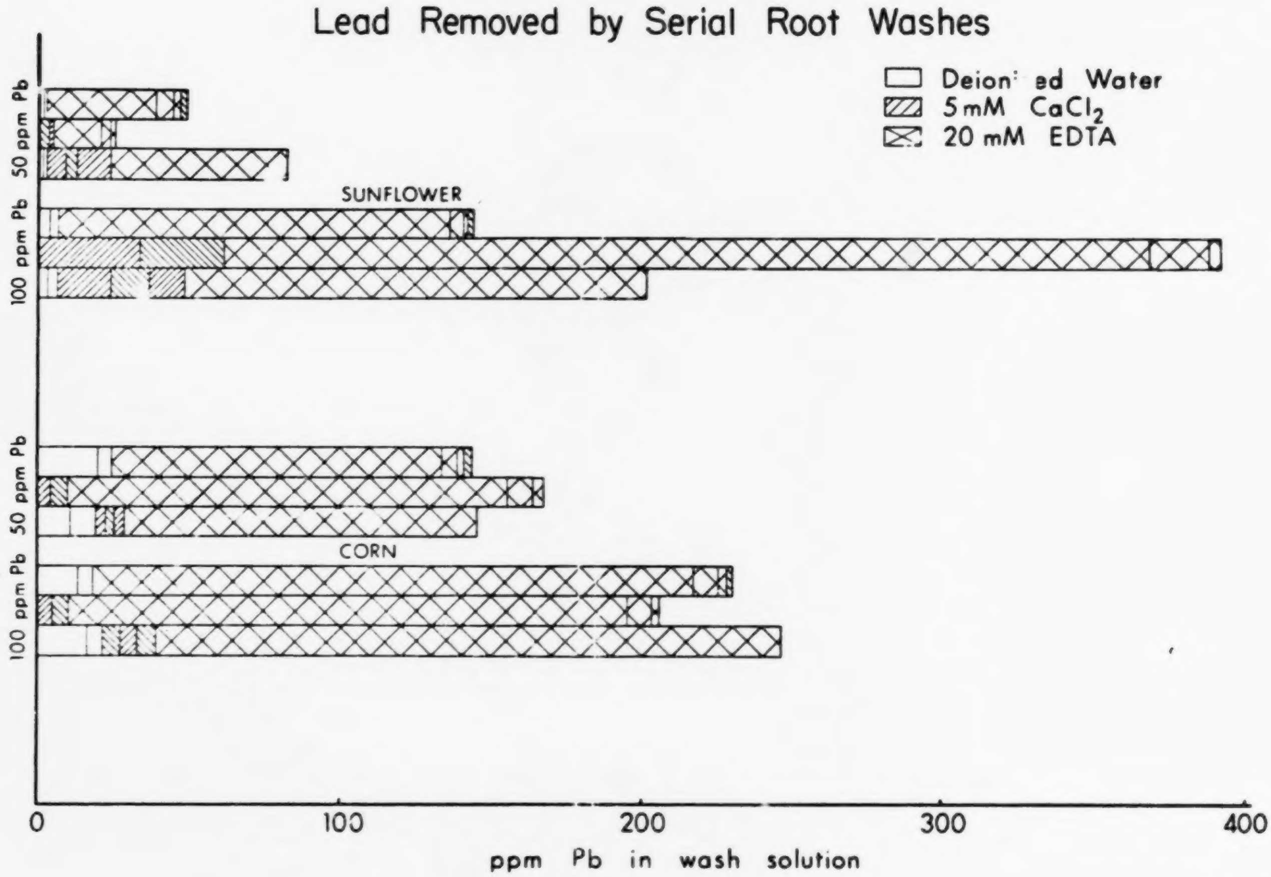

Figure 47. Pb removed from corn and sunflower roots through a series of washes. Wash solutions consisted of deionized water, 5 mM $CaCl_2$, or 20 mM EDTA. Prior to washing, plants were treated for 3 days in either 50 to 100 ppm amended -P Hoagland's nutrient solution. In each case a bar on the graph represents the washing of the roots of 1 plant with a series of 50 ml washes, each lasting 1 hour. The total length of the bar represents the total Pb washed from the roots in the series of washes, and each vertical line separates the individual washes. For example, the top bar, reading from left to right, indicates 2 deionized water washes, 3 EDTA washes, and 1 $CaCl_2$ wash. Since no correction has been made in these data for root weight, it is not possible to make any comparisons of total amounts of Pb washed from the individual plants (See Tables 31 and 32 for this comparison). Prior to Pb treatment, plants were grown in a similar fashion to that described in section IIA.

2) Polarography Experiments Roots from corn plants grown for 2 weeks in nutrient solution were detached prior to the experiment and immersed in a solution containing 2 x 10^{-5} M lead nitrate and 2 mM potassium nitrate. Pulse polarograms were run just before and at different times after introducing the roots into the solution. The solution was kept well stirred at all times except during the recording of the pulse polarograms. The absorption of Pb^{++} by the roots was followed by the decrease in the height of the lead wave (limiting current) in the pulse polarograms.

c. Results and Discussion

1) Root Wash Experiments The experiments reported in Figure 47 and Tables 31 and 32 were conducted for several reasons. First, we wanted to know the amount of lead adsorbed on roots as compared to the amount absorbed. Second, if we were going to wash roots in our uptake experiments, it would be necessary to know what type of wash medium to use. To the second of these questions there seems to be little doubt that EDTA should be used for washing adsorbed lead from roots. Water was almost totally ineffective, while 5 mM $CaCl_2$ was little more so.

The percent lead adsorbed of the total associated with the roots was a surprisingly high 85-95% depending on the species of plant, and the concentration of lead in the treatment solution. With sunflowers, there was on the average slightly more lead absorbed from the 100 ppm solution when compared to the 50 ppm solution, even though the amount adsorbed on the roots was considerably higher in the 100 ppm Pb solutions. With corn there was greater absorption from the 100 ppm Pb solution, but the percentage of absorption remained about the same from the 2 solutions tested.

Table 31. A comparison of Pb washed from sunflower roots to that remaining in roots after washing. Treatments were as indicated in Fig. Treatments listed are W = deionized water, C = $CaCl_2$, and E = EDTA.

Wash treatment	μg Pb associated with 1g oven dry root	Root oven dry weight (g)	Total μg Pb associated with root before wash	Total μg Pb washed from root	μg Pb remaining in root after wash	% Pb washed from root
50 ppm Pb						
WWEEEC	56,032	0.0489	2,740	2,380	360	86.86
CCEEE	67,700	0.020	1,354	1,190	164	87.89
WWCCCE	72,494	0.0469	3,400	3,020	380	88.82
unwashed	50,181					
100 ppm Pb						
WWEEEC	182,979	0.0404	7,385	7,065	320	95.67
CCEEE	279,920	0.0757	21,190	20,735	460	97.85
WWCCCE	224,791	0.0480	10,790	9,850	940	91.29
unwashed	215,827					

Table 32. A comparison of Pb washed from corn roots to that remaining in roots after washing. Treatments were as indicated in Fig. . Treatments listed are W = deionized water, C = $CaCl_2$, and E = EDTA.

Wash treatment	μg Pb associated with 1g oven dry root	Root oven dry weight (g)	Total μg Pb associated with root before wash	Total μg Pb washed from root	μg Pb remaining in root after wash	% Pb washed from root
50 ppm Pb						
WWEEEC	41,307	0.1898	7,840	7,140	700	91.07
CCEEE	35,237	0.2696	9,500	8,480	1020	89.26
WWCCCE	37,026	0.2293	8,490	7,450	1040	87.75
unwashed	32,030					
100 ppm Pb						
WWEEEC	62,761	0.2202	13,820	12,520	1300	90.59
CCEEE	58,418	0.1966	11,485	9,965	1520	86.77
WWCCCE	68,182	0.2123	14,475	12,475	2000	86.18
unwashed	------					

2) Polarography Experiments Due to the maxima developed on the lead wave after the addition of roots to the solution (Figure 48), the limiting currents were measured at -0.42 V by back extrapolation of the linear region between 0.70 and 0.90 V. A plot of the limiting current versus time is shown in Figure 49 and the shape of the plot is in general agreement with the dual absorption phenomena at the roots. Initially, the uptake is predominantly due to the ion exchange adsorption which ceases with time and further uptake would then be only due to the metabolic absorption.

Addition of $Ca(NO_3)_2$ to the solution after the above 2 hour absorption experiment, released Pb^{++} from the roots back into the solution. The pulse polarogram recorded at 2 hours after adding $Ca(NO_3)_2$ is also shown in Figure 49 for comparison. Before the addition of $Ca(NO_3)_2$, only about 8% of the total lead added was in solution but after the addition, about 80% of it appeared in the solution. This experiment confirms the ion exchange property of corn roots indicated by the time dependence study of absorption (Figure 49).

Approximate caluclations from the above experiments show that at least 1.30 μmoles of Pb^{++}/g of fresh corn root can be held by the ion exchange process. In determining the lead uptake by plants, this Pb^{++} held to the outer surface of the roots can introduce large errors unless eliminated or properly corrected. Elimination can be achieved by ion exchange competition as shown by Ca^{++} in our experiment described above or by complexation. Complexation with reagents like EDTA reduces the free lead ion concentration in the solution and consequently decreases the extent of lead exchange by roots. Preliminary studies showed that 10 mM EDTA can be quite effective in removing exchanged lead on the roots.

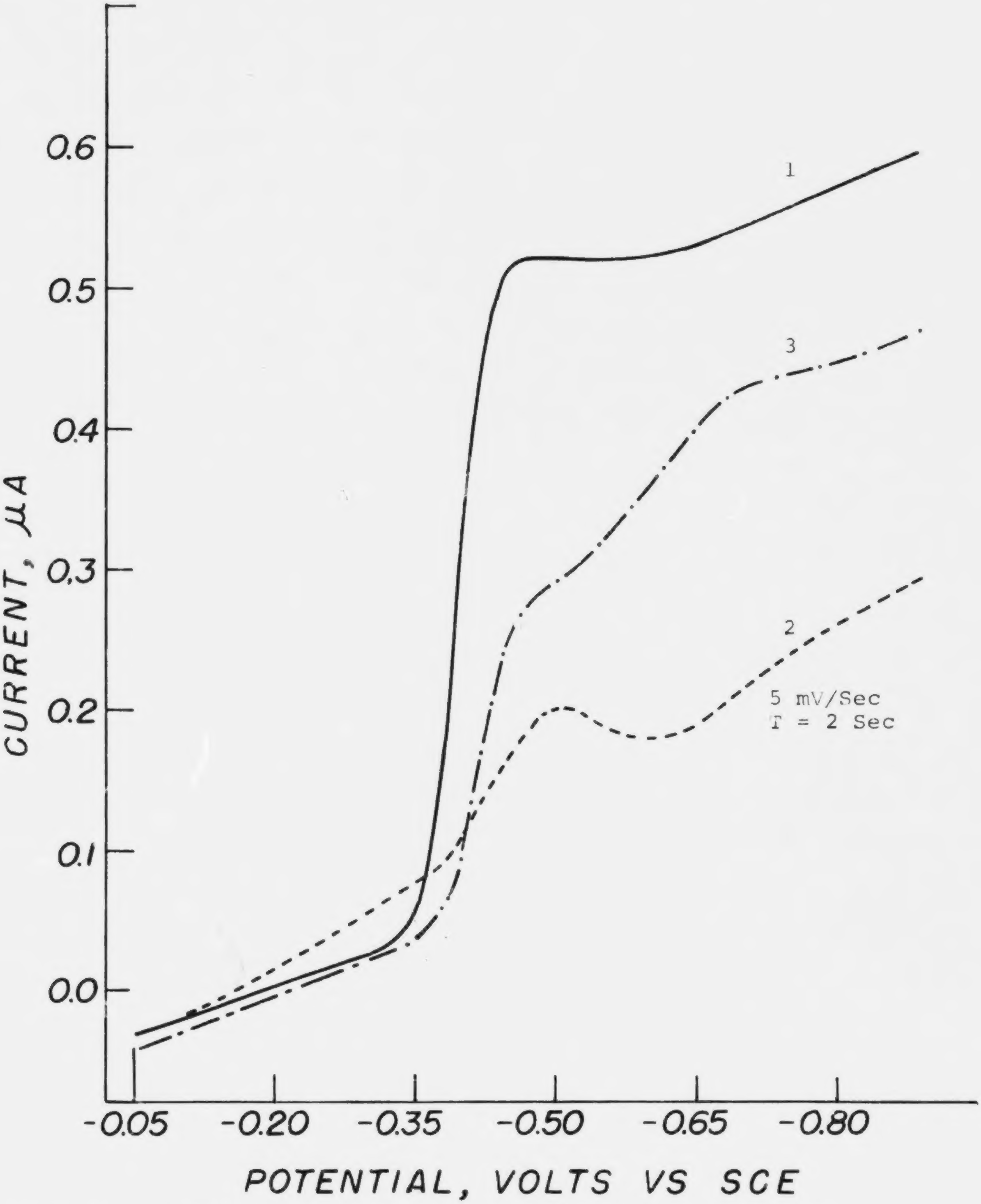

Figure 48. Pulse Polarograms of $2 \times 10^{-}$ M Pb^{++} in 2 mM KNO

1. Before adding corn roots
2. 2 hrs after adding corn roots (0.70 g freshweight)
3. 2 hrs after adding Ca(NO) to the above solution with roots - Ca^{++} concentration 0.069M.

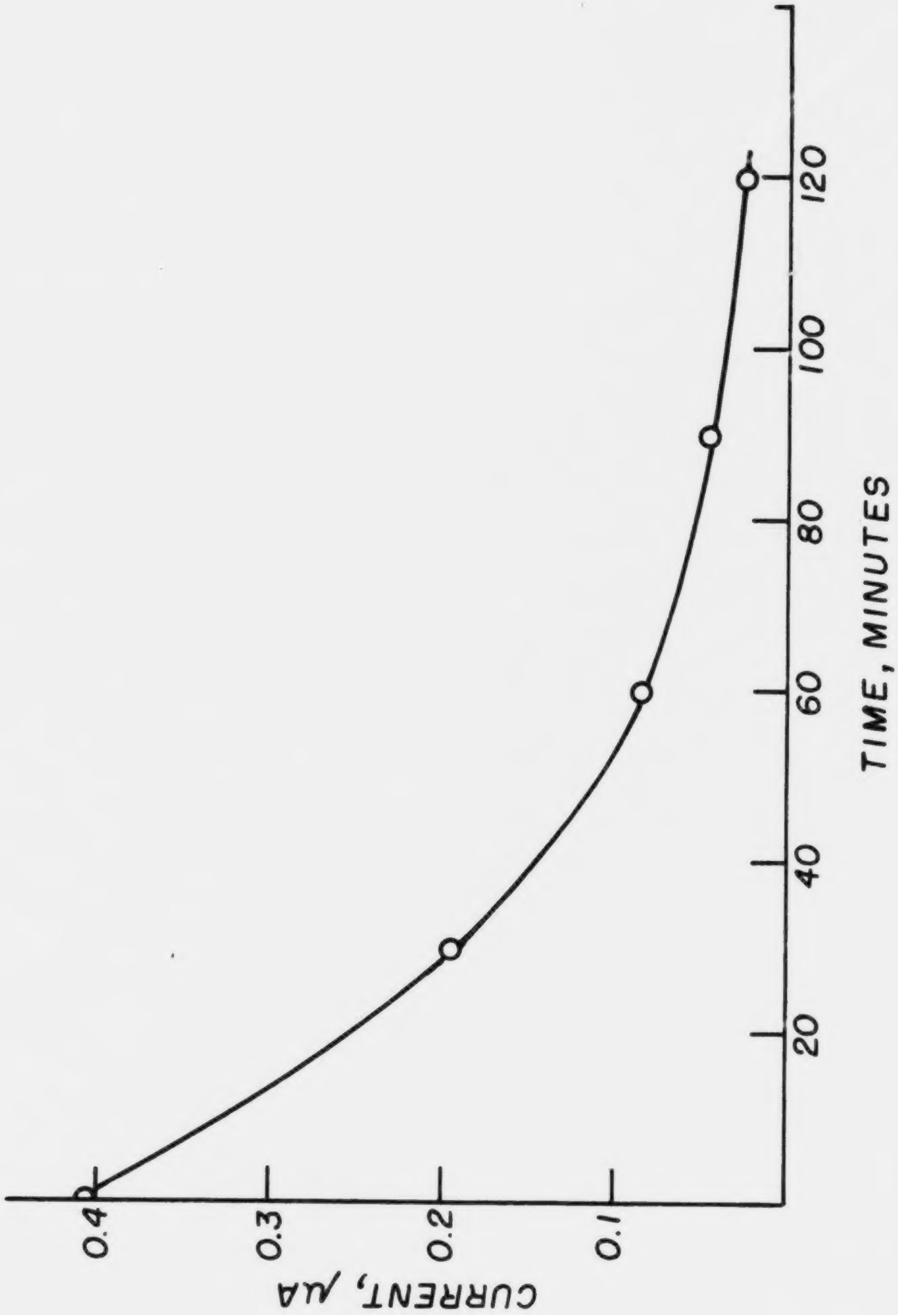

Figure 49. Effect of Freshly-Detached Corn Roots with Time on the Pb^{++} Concentration in Solution as shown by the Limiting Current in the Pulse Polarographic Wave.
Solution - 60 ml of 2×10^{-5} $\underline{M}$ $Pb(NO_3)_2$, 20 m$\underline{M}$ KNO_3
Roots - 0.70 g (fresh weight)

2. The Association of Root Exudate and Lead

a. Background. During their normal operation, plant roots absorb water and nutrients from their surroundings and at the same time they also exude or release a wide range of organic compounds. These exudates include sugars, amino acids, proteins, vitamins, enzymes, and miscellaneous other compounds (Rovira, 1969). Corn plant roots in particular were known to exude at least 15 amino acids besides sucrose, fructose, and glucose (Frank, 1954; Ivanko and Javor, 1970; Rovira, 1969; Kandler, 1951). Continuous accumulation of the exudates around the roots can significantly alter the nature of the soil and soil solution around the roots. For example, the metal ions in the soil solution surrounding the roots can be complexed or precipitated by the exudate species. It is also possible that these exudates react with the metal ions held to the soil particles and mobilize them into the soil solution. Thus, the root exudates can play a beneficial or detrimental role rowards the plants depending on whether they are increasing or decreasing the nutrient and toxic metal ion concentrations surrounding the roots. The studies with corn root exudate and lead ion reported below show that the exudate acts both as a complexing agent and also as a precipitating reagent for Pb^{++} depending on the amount of lead present in the solution.

b. Procedures. The corn root exudate used in this work was obtained by the following procedure. Fifteen corn plants were grown for 8 weeks in quartz sand culture with Hoagland nutrient solution. The roots intact with the plants were washed with running deionized water for 24 hours and the exudates collected during the next 48 hours in 4 liters of deionized water circulated around the roots. The 4-liter solution was then reduced to 100 ml by low temperature distillation at 40°C and filtered to remove the insoluble residue at the bottom and the material suspended in solution. Analysis showed that the above exudate concentrate contains phenols (28 μg/ml catechol equivalent), amino acids (48 μg/ml leucine equivalent), carbohydrates (90 μg/ml glucose equivalent) and proteins (probably <20 μg/ml). In the rest of the report, this exudate concentrate will simply be referred to as exudate alone.

c. Results and Discussion A significant shift in the half-wave potential of the lead wave in the pulse polarograms and also a decrease in the wave height was caused by the exudate. Addition of 1 ml of exudate to 10.3 ml of 0.02 M KNO_3, 1.45×10^{-6} M $Pb(NO_3)_2$ solution shifted the lead wave by about 1 volt to more negative potentials (Figure 50). A shift in a polarographic wave is normally associated with complexations of the metal ion for a reversibly reduced metal ion such as lead. In addition, adsorption of a species on the electrode surface can cause the wave to shift by occlusion on the surface. A simple complexation reaction alone cannot explain the observed shift as it would require the formation of a complex with an extremely high stability constant. Calculations made by using the equations for the half-wave potentials ($E_{1/2}$) for the reversible reduction of a metal ion and its complex (Meites, 1965) show that for a divalent metal ion the complex should have a stability constant of at least 10^{30} to produce the shift in $E_{1/2}$ by 1 volt. In the calculation, the concentration of the complexing ion (ligand) was taken as 1 M and the metal to ligand ratio in the complex as 1. If the ligand concentrations were lower than 1 M, the calculated stability constant would be even greater than 10^{30}.

Amino acids in the exudate will definitely complex part of most of the lead ions present in solution but the available data on the stability

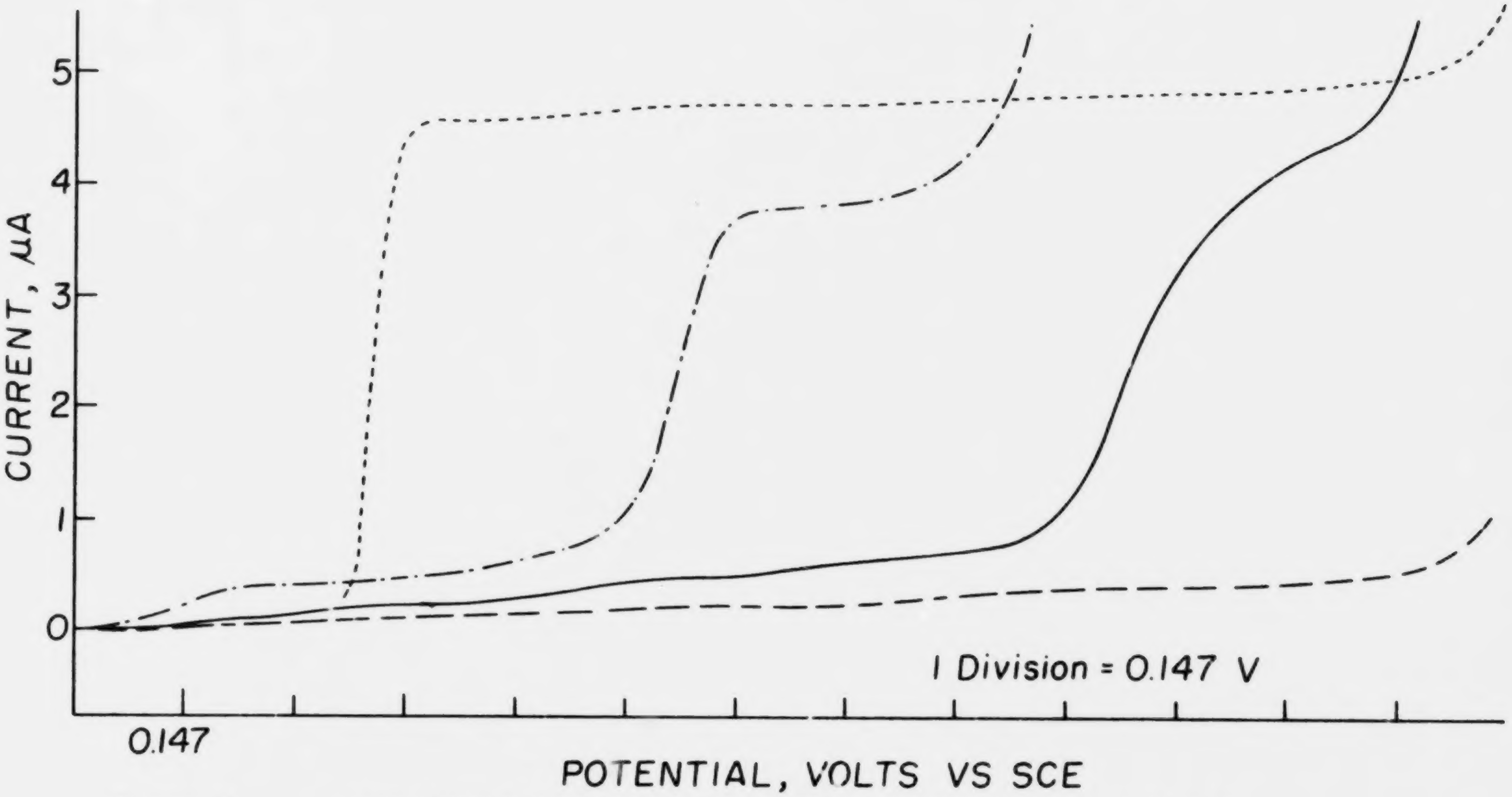

Figure 50. EFFECT OF CORN ROOT EXUDATE AND EDTA ON THE PULSE POLAROGRAMS OF LEAD IN 20 mM KNO_3

1. — — — 10 ml of 20 mM KNO_3
2. - - - - - - - - Solution 1 + 0.3 ml 5 mM $Pb(NO_3)_2$
3. ——— Solution 2 + 1 ml Corn Root Exudate
4. —-—-— Solution 3 + 0.2 ml glacial Acetic Acid + 0.4 ml 0.2 M Na_2 EDTA

constants of metal-amino acid complexes do not support the formation of lead-amino acid complexes with stability constants much higher than 10^{15}. Data on the stability of metal-protein complexes are scarce but it appears that their stability constants can be at most 2 or 3 orders of magnitude greater (if at all) than the metal ion complex of the amino acid that constitute the side chain residues in the protein molecule (Vallee and Wacker, 1970). There are a few metal-protein complexes reported in literature (Kolthoff et al., 1954; Davis et al., 1962) that have exceptionally high stabilities ($K > 10^{25}$) but in all probability, lead-protein complexes are not that stable. In any event, the diffusion coefficients of lead-protein complexes would be much lower than that of hydrated lead ion, so such complexes could not yield the observed currents. Clear evidence to the fact that lead-exudate complex is not of extreme high stability ($K \sim 10^{30}$) is given by the following experiment.

Pulse polargograms were recorded before and after addition of EDTA to a solution containing lead and exudate. It was found that the lead wave shifted to less negative potentials suggesting the lead-exudate complexes must have a stability constant $\lesssim 7.94 \times 10^{17}$, the value for lead-EDTA complex (Stillen and Martell, 1964). Figure 50 includes the pulse polarograms obtained before and after adding EDTA and HOAC to a solution containing lead and exudate.

Adsorption of a species on the electrode surface can prevent an electrode reaction from taking place until a potential is reached where the species desorbs from the surface. In other cases, the electrode reaction can proceed through the adsorbed layer at sufficiently negative cathode potentials. Adsorption effects of proteins at the dropping mercury electrode were reported by several workers. A summary can be found in the monograph on Polarography by Brazina and Zuman (1958). The potential region of adsorption and the extent of it depends on the nature of the protein (or any other adsorbing species in the exudate) and also on the solution composition. To complicate matters further, the electrode reaction in the presence of exudate is irreversible, especially in the presence of an adsorbed material, and the stability constant calculations made above are not valid for such a system. The irreversibility of an electrode reaction would shift the reduction waves to more negative potentials than expected in the case of a reversible reaction and it offers a reasonable explanation for the data observed.

Comparison of the lead wave heights in the pulse polarograms in Figure 50 shows that the addition of exudate caused a drop in wave height by about 20% of the original lead wave in 20 mM KNO_3. Also the addition of EDTA and acetic acid did not restore the wave height suggesting that it is not simply due to metal ion complexation with large molecules like protein. It appears as if 20% of the lead in solution reacted with exudate species and became available to the electrode. The experiment reported below shows that there are at least three forms of lead present in the solution with the exudate.

To a 10 ml solution containing 20 mM KNO_3 and 2 ml exudate, increasing amounts of lead nitrate were added while recording the pulse polarograms after each addition. Noticeable changes became apparent in the pulse polarograms when the lead nitrate added amount to 10^{-4} M in the solution. Typical polarograms obtained on either side of this concentration are shown in Figure 51. At low concentrations of lead, only one wave was observed at -1.40 V while at high concentrations two more waves appeared at -0.47 and -0.91 V (Figure 51). Approximately in the same concentration

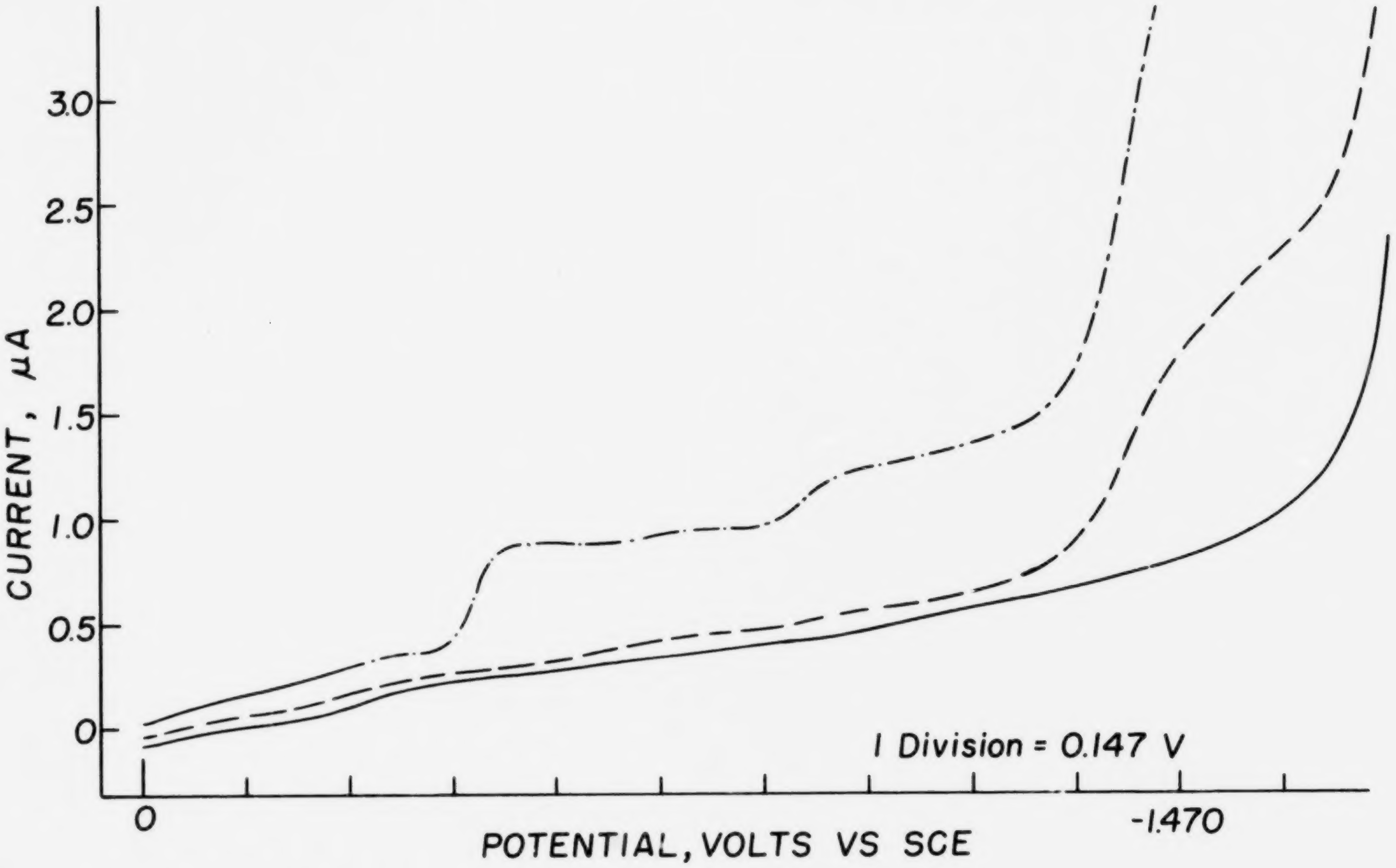

Figure 51. EFFECT OF LEAD CONCENTRATION ON THE PULSE POLAROGRAMS OF LEAD IN CORN ROOT EXUDATE SOLUTION (1:10 dilution) WITH 20 m$\underline{M}$ KNO_3

Apparent Lead Concentration in Solution

——— 0 M
– – – 4.95×10^{-5} M
–·–·– 2.47×10^{-4} M

range where the latter two waves appeared ($\gtrsim 10^{-4}$ M), the solution showed some turbidity which increased with further addition of lead nitrate. The actual identity of the precipitate formed is not known but it dissolved in 0.2 M acetic acid and also in 4 mM EDTA. Though the lead wave -1.40 V showed rather steady increase in its height with the amount of lead nitrate added to the solution, the other two waves increased only to a small extent

The results of the above experiments indicate that the corn root exudate probably can solubilize the lead in an insoluble form (e.g. lead phosphate, lead carbonate, etc.) and mobilize the immobile lead (e.g. Pb^{++} on soil particles). Furthermore, in solutions with high lead concentrations, the exudate may tend to reduce the soluble lead concentration by precipitation. The extent to which the exudate will be involved in the above reactions depends on the relative concentrations of lead and the exudate species. The above comments made about lead and corn root exudate are in general applicable to other metal ions (especially the heavy metal ions) and other plant root exudates as well. Some of the exudate reactions mentioned above are under futher investigation.

3. The Uptake of Lead and Cadmium by Organisms

a. Corn

1) Background A previous report (Miller and Koeppe, 1971) observed large amounts of lead uptake through roots of corn grown in sand culture. This lead accumulated in leaves of phosphate-deficient plants to a level comparable to low-grade lead ore. However, while this study did illustrate that lead was readily taken up through roots and translocated to leaves, the concentrations of lead in the sand medium were far in excess of

what the plant would encounter under natural conditons. In this section are reports of uptake and accumulation of lead and cadmium from solutions containing much lower concentrations. Even these concentrations (up to 100 ppm) are probably much higher than plants will encounter in nature. However, we must know something about the rate of lead (or other trace elements) uptake and its deposition within the plant, and analytical procedures dictate that concentrations of lead be relatively high for determination by atomic absorption. Thus, in our initial studies we have chosen to work with moderate concentrations of lead in our uptake solutions so that the amount of plant material to be prepared for lead analysis can be kept to a minimum.

2) Procedure. Preliminary - Several series of experiments were run before determining the best technique for treating plants grown hydroponically with trace elements. Questions relating to solution volume, plant age and size, ratio of nutrient solution to plant mass, bacterial and/or fungal growth, numbers of plants/treatment, solution pH, nutrient solution temperature, solution mixing, solution aeration, and how to chelate the iron in the nutrient solutions had to be answered before anything other than very preliminary experiments could be attempted. For the most part our preliminary scheming and planning directed us along correct paths relating to these potential problem areas. The problems of solution pH and iron chelate, however, were worked out only through experimentation involving weeks of plant growth and development.

Finalized techniques. Corn is germinated in a germination cabinet (29 ± 0.05°C) and then carefully placed into polyethylene cups. These cups are made by melting the tops of 2 polyethylene stoppers together, removing the bottom from one stopper, and perforating the bottom of the other.

The roots of the germinated corn seedlings are placed through the perforated end of the cup which is placed into styrofoam supports (each holds 42 cups) which float on a 12 l nutrient medium (Hoagland's solution + Fe^{+3} citrate) contained in a polyethylene dish pan. The solution is constantly aerated with an airstone and the pH is maintained between 3.5 and 4.0. The entire container is covered with black polyethylene to prevent light penetration.

After 2 weeks, the cup-plants are transferred to plexiglass holders (12 plants/treatment) above 12 l treatment solutions in polyethylene dish pans. Each dish pan is fitted with a manometer that is sensitive to solution volume changes of 50 ml or more (less than 1% of the total volume). Solution volumes are corrected each day as is the pH. Again, all containers are aerated through airstones. Appropriate precautions are taken to insure that no trace element contamination occurs. All equipment is acid washed between experiments and containers with high trace element concentrations are only used from experiment to experiment for such high concentrations.

After appropriate treatment periods plants are removed and placed into beakers containing 10^{-4} M EDTA (50 ml/plant) and the roots are washed twice in the EDTA solutions for periods of 30 minutes. Subsequently, plants are divided into sections to be analyzed, air dried, oven dried at 105°C. desiccated and weighed, ashed overnight at 450°C, and the ash taken up in 3 N HCl. Trace element analyses are made by atomic absorption.

3) Results and Discussion

Effects of Phosphate Deficiency With the exception of roots treated for one week, phosphate deficient (-P) plants accumulated as much or more lead on a ppm basis as did phosphate sufficient (+P) plants

(Figure 52). Toxic effects of lead were observed as a slight stunting of growth in both phosphate treatments. In all samples there was less lead accumulation in plants treated with 100 ppm than in plants treated with 50 ppm. This was not observed in the +P plants and most likely is due in the -P plants to a detrimental effect of lead that did not show up as an observable toxic symptom.

Accumulation of Lead in Different Tissues   Generally there was more lead accumulation in leaves initiated before lead treatment (old leaves) than in those initiated after the start of lead treatment (new leaves) (Figure 52). Concentrations of lead associated with roots were in all cases higher than those in leaves, but this concentration does include appreciable adsorbed lead in addition to that actually within the root. Estimates of this proportion to be given later in this section indicate that approximately 85-90% of the lead associated with the root is only adsorbed. If only 15% of the lead reported associated with the root in Figure 52 is in the root, then root concentrations are actually not tremendously higher than those in the leaves.

Form of Lead Depostion Within Corn   Corn leaves containing more than 4000 ppm Pb were air dried and sent to the laboratories of Walter McCrone for analysis. Scans of portions of the tissue were made with the electron microprobe for K, C, Pb, and P. Lead was distributed throughout most of the tissue as distinct almost point sources, and coincided almost directly with the phosphate. Dr. McCrone's analysis indicated the likelihood of Pb-P compounds, some of which could be seen as particles.

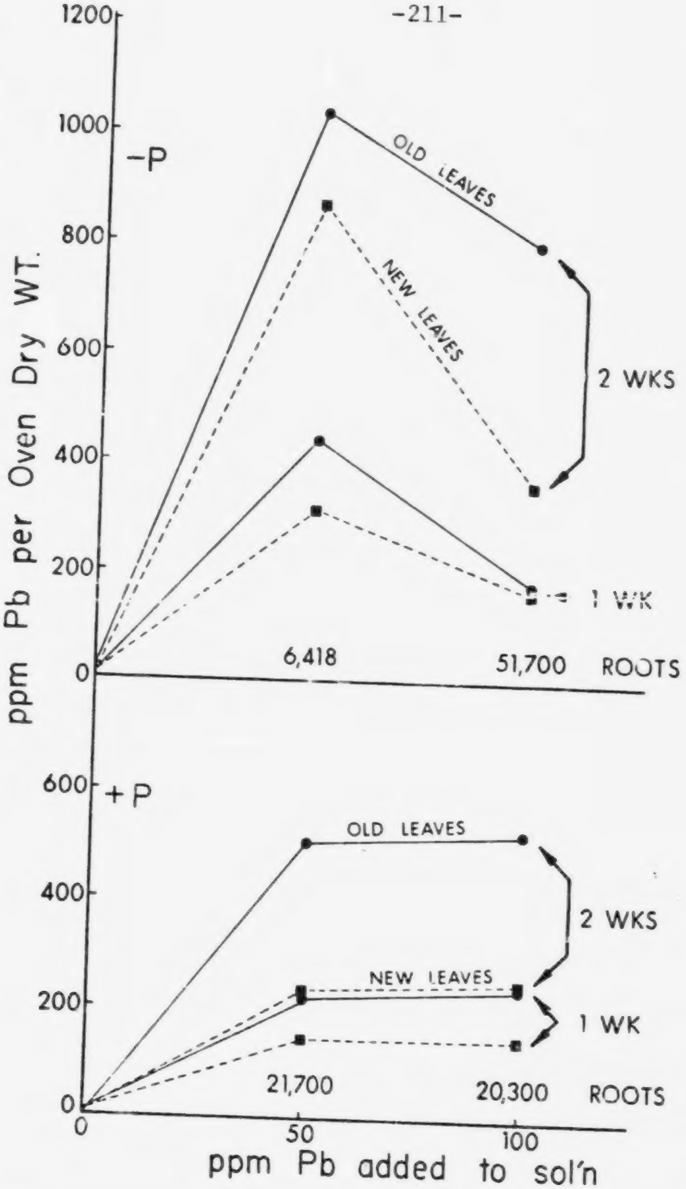

Figure 52. The uptake of Pb by corn from Pb amended hydroponic solutions. Plants were grown for 2 weeks, treated for 1 or 2 weeks with Pb, and then harvested as described in section B-1 with the exception that Fe^{+3}-EDTA was used, pH was allowed to rise from 3.5 to 6.5 during the 2 weeks treatment period, and the roots were washed with water only. Results designated -P were obtained from plants grown since germination in the complete absence of phosphate, while those designated +P had a continuous source of P. At the time treatment was initiated (2 weeks), P deficient symptoms were evident in the -P plants. The length of treatment is represented on the graph as 1 WK or 2WKS. Numbers given for roots are the ppm Pb associated with roots grown in 50 to 100 ppm Pb solutions for 2 weeks. New leaves are those that were initiated after the beginning of Pb treatment, while old leaves are those that were initiated before Pb treatment.

Further analyses are being planned with Dr. McCrone to determine if there is any difference in the form of lead between the analyses already carried out on dried tissues, and the form in living tissues.

Cadmium Uptake by Corn Cadmium was found to be readily taken up by corn, but quickly killed it at growth media concentrations of 50 ppm and more. Figure 53 presents data on concentrations of cadmium found in corn tissues after 1 and 2 weeks treatment. As with lead, more cadmium was accumulated in old leaves as compared to younger leaves, and amounts of cadmium associated with roots were considerably more than concentrations found in leaves. Most probably at least some of the cadmium was adsorbed to roots since again these roots were only washed with water before analysis. It is interesting to note, however, that concentrations of cadmium associated with roots were much less than those for lead. Data to be presented later in this report indicate far less binding of cadmium to membranes of mitochondria, which could also be the case for cadmium binding to roots.

b. Lead Uptake by Selected Tree Seedlings

1) Background Although considerable research has been done concerning contamination of vegetation by lead, there are many unanswered questions. Research on lead uptake by plants is incomplete, but seems to indicate that plants take lead up from the soil but generally only small amounts. The factors affecting and controlling uptake and translocation are unknown.

The present study examines the uptake of lead by selected tree species from a soil source and attempts to answer the following questions.

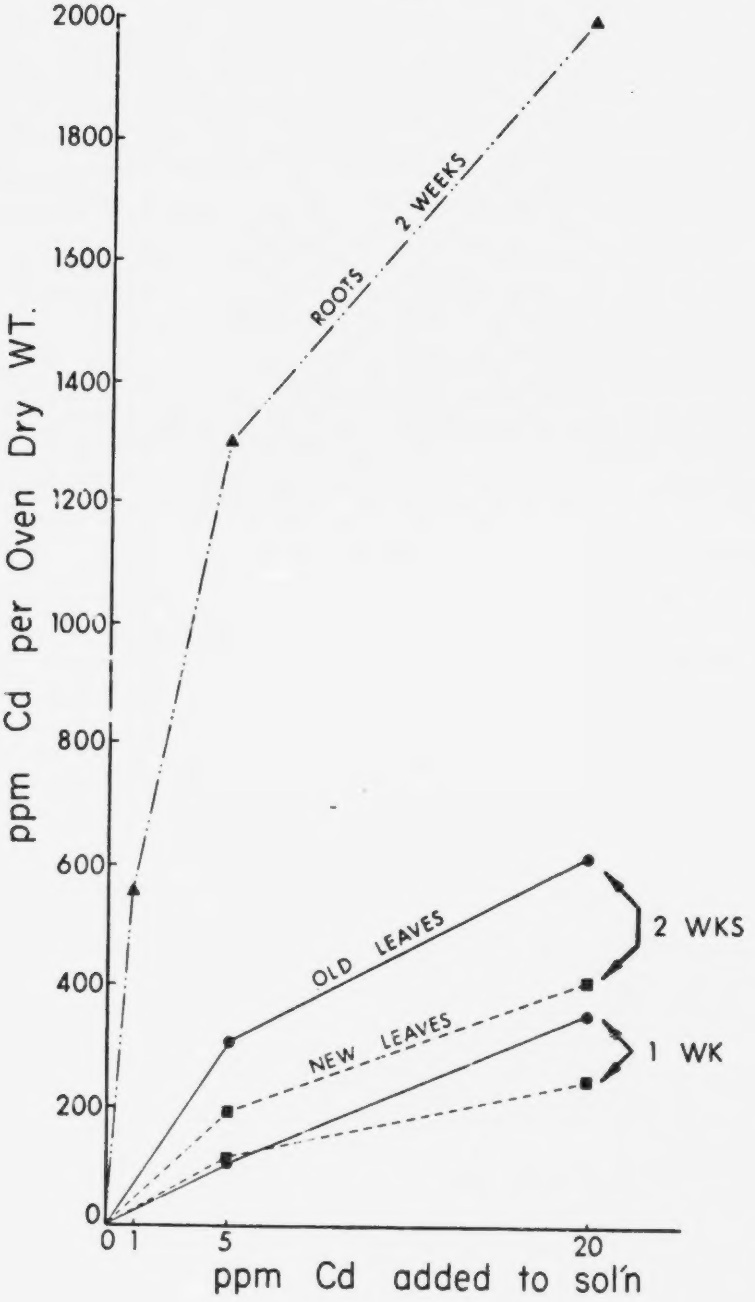

Figure 53. The uptake of Cd by corn from Cd-amended hydroponic solutions. Plants were grown for 2 weeks, treated for 1 or 2 weeks with Cd, and then harvested as described for Pb in Figure 52 The length of treatment is represented on the graph as 1 WK or 2 WKS.

Can tree species accumulate significant concentrations of lead in roots, stems, and leaves over a normal growing season? What is the effect of soil lead level on uptake? What is the effect of the species of tree on lead uptake? What is the overall effect of general soil factors on lead uptake?

2) Procedures The tree species chosen for the experiment were two-year-old nursery stock and selection was based on growth rate, planting applications, and whether the species was deciduous or evergreen. The species chosen were cottonwood (*Populus deltoides* Bartr.), red oak (*Quercus rubra* L.), white oak (*Quercus alba* L.), yellow poplar (*Liriodendron tulipifera* L.), black locust (*Robinia pseudoacacia* L.), shortleaf pine (*Pinus echinata* Mill.), and loblolly pine (*Pinus taeda* L.). Autumn olive (*Elaeagnus umbellata* Thunb.), a fast growing, shrubby species commonly used as an ornamental, was also used in the experiment.

Soils used in the study were Plainfield sand (a typic udipsamment), Grantsburg silt loam (an ochreptic fragiudalf), and Flanagan silt loam (an aquic argiudoll). The soil was secured from areas remote to major lead sources and only the upper six inches of the profile were taken. Potting mixtures consisted of two-thirds soil and one-third vermiculite. Soil characteristics are shown in Table 33.

TABLE 33.

Soil Characteristics

Soil Type	pH	Cation Exchange Capacity m.e./100 g	Total Lead ppm	Available Phosphorus lbs./acre
Grantsburg Silt Loam	5.2	11.3	3.0	20
Flanagan Silt Loam	5.9	21.0	6.2	38
Plainfield Sand	5.8	8.9	4.7	18

Plants of uniform size were selected, transplanted into pots, and placed in the greenhouse under artificial light. Temperature was maintained in the greenhouse at 80° F. ± 10° F., and day length was sixteen hours. All plants were watered daily with deionized water having a lead content of less than 1 ppm. After the plants were growing well, the experiment began.

Five lead levels were established in each soil type by adding a solution of lead chloride ($PbCl_2$) in deionized water to each pot. Lead chloride was applied to duplicate as well as possible the typical form and solubility of lead emitted into the environment through the combustion of gasoline containing tetraethyl lead. The chief constituents of the inorganic lead compounds leaving the exhaust system are PbClBr, two forms of $NH_4Cl \cdot 2PbClBr$, and $2NH_4Cl \cdot PbClBr$ (Hirschler et al., 1957). Lead bromochloride (PbClBr) appears to be an ordered structure which is stable at ordinary temperatures and isomorphous with $PbCl_2$ and $PbBr_2$ (Calingaert et al., 1949). Lead levels used were: 0, 75, 150, 300 and 600 ppm Pb. This range of lead levels was selected to include both present-day soil levels and future levels which may eventually be reached by the continued addition of lead to the environment.

Two soil phosphate levels were used: the natural level of the soil (Table 33) and a fertilized level. The fertilized level was established by the addition of phosphate in the form of monocalcium phosphate $Ca(H_2PO_4)_2 \cdot H_2O$ at a rate of 300 pounds of phosphate per acre.

The plants were then grown for ninety days, simulating the peak period of the growing season. At the end of the ninety-day period, the plants were harvested and separated into leaf, stem, and root material. Leaves and stems were not washed, but root material was washed for thirty minutes in deionized water and rinsed three times in order to remove as much soil and lead accumulated on the surface of the root as possible.

Analysis of the samples was by atomic absorption spectroscopy. The samples were prepared for analysis by the following procedures. Samples were oven-dried for two hours at 105°to 110°C. A 1.00g sub-sample was weighed and placed in a 30 ml Vycor crucible. The samples were then dry ashed at 450°C. for five hours. The sides of the crucible were washed with 20 ml of 6N HCl diluted to 3N with deionized water and the samples were digested for 3 hours on a steam bath. The samples were then transferred to polyethylene bottles and diluted with deionized water to 20g. All containers and glassware used in the procedure were leached prior to use with 3N HCl to remove any surface impurities.

A second experiment attempted to isolate the effect of soil phosphate on lead uptake by tree seedlings. Two species, yellow poplar (*Liriodendron tulipifera* L.) and loblolly pine (*Pinus taeda* L.), were grown in vermiculite to which nutrient solutions and lead were added daily. Leachate from several pots was collected and analyzed for lead content in order to determine if lead was accumulating in the vermiculite. To minimize accumulation, the pots were flushed biweekly with EDTA.

Two levels of phosphate were used (0 and normal Hoagland nutrient solution level) and five levels of lead (same as soil experiment) were established and maintained in the pot cultures. Environmental conditions were the same as in the soil uptake study.

The plants were harvested after sixty days and separated into leaves, stems, and roots. From this point, procedures identical to those in the soil study were followed.

3) Results and Discussion After completion of the 90-day study period, all species exhibited a significant ($P \leq 0.005$) concentration of lead in leaves, stems, and roots at all application levels of lead and phosphate and in all soil types. No significant ($P \leq 0.05$) differences in lead uptake were found between soil types although differences were anticipated due to the wide range of physical and chemical properties between the soils used in the study. The fact that no significant differences were noted does not substantiate that soil type has no effect on lead uptake but does imply that soil type has less effect than was anticipated. Plants grown in all three soil types with high rates of applied lead (300 and 600 ppm) and normal phosphate levels (unfertilized began to show visual phosphate deficiency symptoms after 60 days. Plants grown under conditions of added phosphate showed no visual symptoms of deficiency at any level of lead application. The phosphate deficiency is probably a result of the formation of insoluble lead phosphate precipitates both in the soil system and in the plant (Koeppe and Miller, 1970).

In order to simplify presentation of the data, species have been grouped into ranges of lead uptake. There are no significant differences ($P \leq 0.05$) within a specific group but differences between groups are significant ($P \leq 0.005$). Uptake values of all three soil types were averaged before plotting since there were no significant differences in uptake between soil types.

Results of leaf lead analyses are shown in Figure 54. Two ranges of lead uptake are shown. Range I contains shortleaf pine (SP), loblolly pine (LP), autumn olive (AO), and black locust (BL). All of these species are relatively fast growing and grew well under greenhouse conditions. Leaf lead levels of over 100 ppm were found in these species when grown in soils with a lead level of 600 ppm. The second range contains white oak (WO), red oak (RO), yellow poplar (YP), and cottonwood (CW). These species had significantly lower uptakes at all soil lead concentrations (Figure 54). It was expected that the two species of oak would show low uptake rates since they are generally slow-growing species but cottonwood and yellow poplar have growth rates comparable with species in range I. The lower uptake by these two species is probably the result of a light scorch which affected these two species in particular during the study period.

Also shown in Figure 54 are uptake ranges for the same species and soils under conditions of added phosphate. The same two groups are apparent but have respectively lower uptakes when compared with uptake ranges without added phosphate. The differences are significant at the 0.5% level. Again, this is probably a result of the formation of insoluble lead phosphate which is not available for plant uptake.

Results of the stem analyses are shown in Figure 55. Three uptake ranges are evident. Range I includes shortleaf pine and white oak. Range II consists of black locust, yellow poplar, and autumn olive while range III contains loblolly pine, red oak, and cottonwood. Although three ranges are identified, the grouping of species within ranges is still quite comparable with species groups found in the leaf analyses with one exception. White oak, found in the lower range as expected in the leaf analyses, now appears in the high uptake range in the stem analyses. This may be due to a high uptake rate by this species but a slow translocation from stem to leaves. Another factor which may affect this is the indication by Kuntz and Riker (1955) that translocation of injected materials in oak species occurs not only upward but also downward which would tend to hold materials such as lead in the root and stem for longer periods of time.

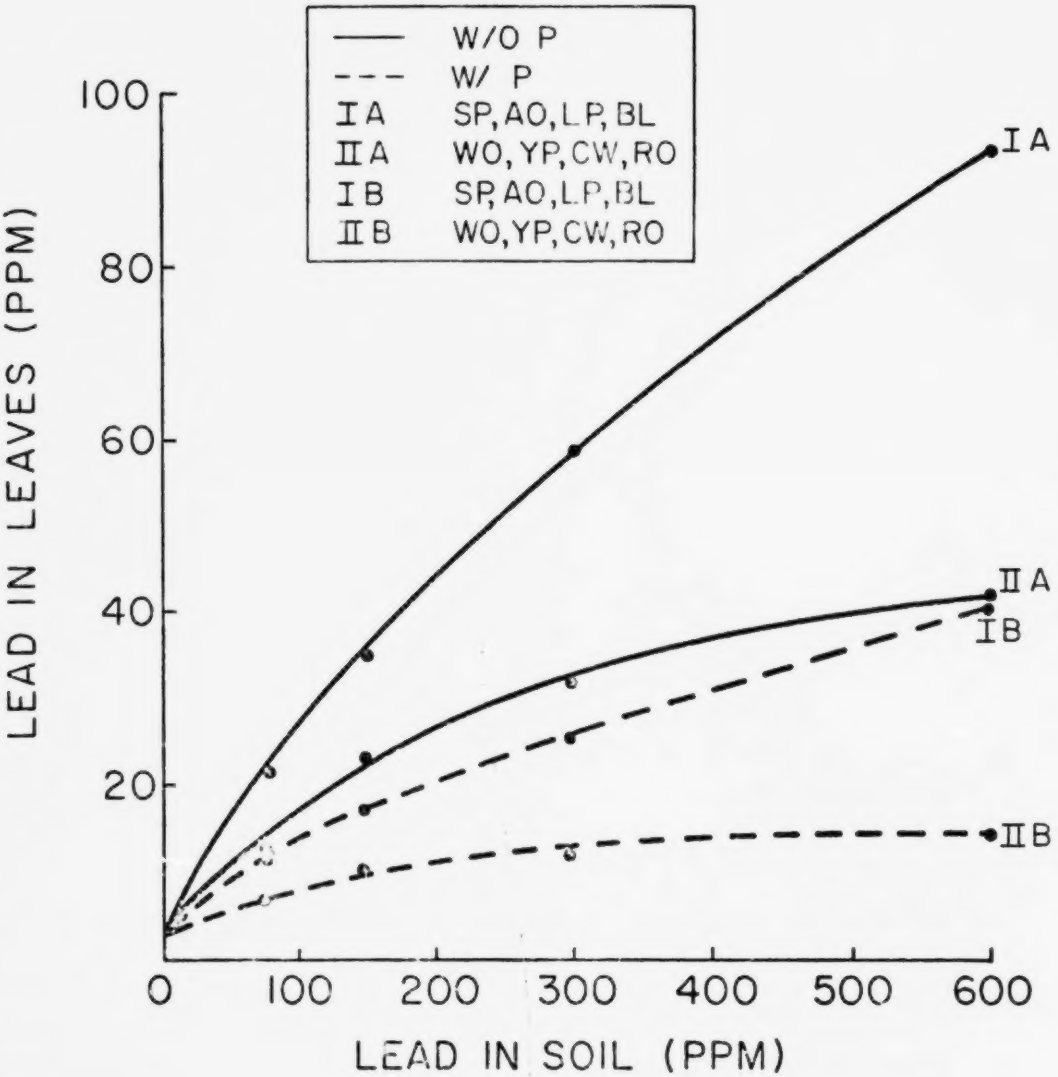

Figure 54. Leaf Lead Concentrations Derived from a Soil Source

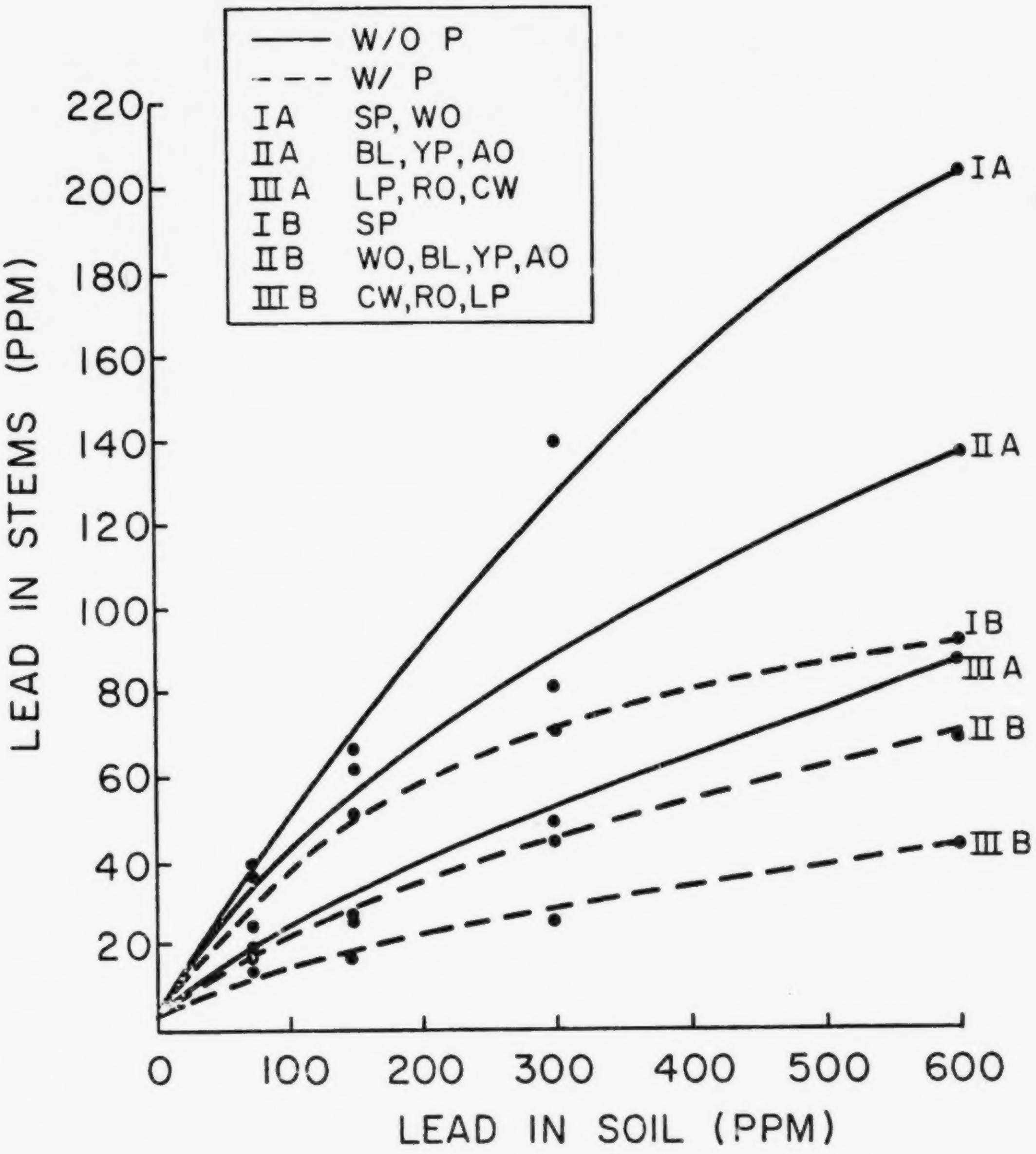

Figure 55. Stem Lead Concentrations Derived from a Soil Source

As with the leaf results, under conditions of added phosphate, a significant decrease ($P \leq 0.005$) in lead uptake is noted. The same uptake groups are evident except for white oak moving from range I to range II.

Root analyses data are shown in Figure 56. Three ranges of uptake are again identified but grouping within the ranges is considerably different. Range I consists of loblolly pine and autumn olive; Range II, shortleaf pine, white oak, and yellow poplar; and Range III, cottonwood, red oak, and black locust.

Extremely high concentrations of lead were found in and on the washed root systems of all species. Results were quite variable due to the difficulty encountered in washing the root material. By simple water washing, described under procedures, much surface lead and soil contamination is removed very easily from some species such as yellow poplar while other species with very fibrous, matted root systems such as autumn olive are difficult to wash with any success. Due to this variation, the data presented do not adequately answer the question as to how much lead is actually in the root material.

With the addition of phosphate, a significant decrease ($P \leq 0.005$) in root lead level is again evident. The same uptake groups are retained except black locust moves from range II to range III.

Results of the second experiment indicate a significant ($P \leq 0.005$) uptake of lead by both loblolly pine and yellow poplar at all levels of lead and phosphate application. Results are shown in Figures 57, 58, and 59. Plants grown in solution cultures without phosphate exhibited severe phosphate deficiencies after forty days of the sixty-day growth period. Plants grown in nutrient solutions with normal phosphate levels had significantly ($P \leq 0.005$) lower lead uptakes at all levels of applied lead than did those grown under phosphate deficient conditions. Loblolly pine had a significantly ($P \leq 0.005$) higher uptake under all conditions than did

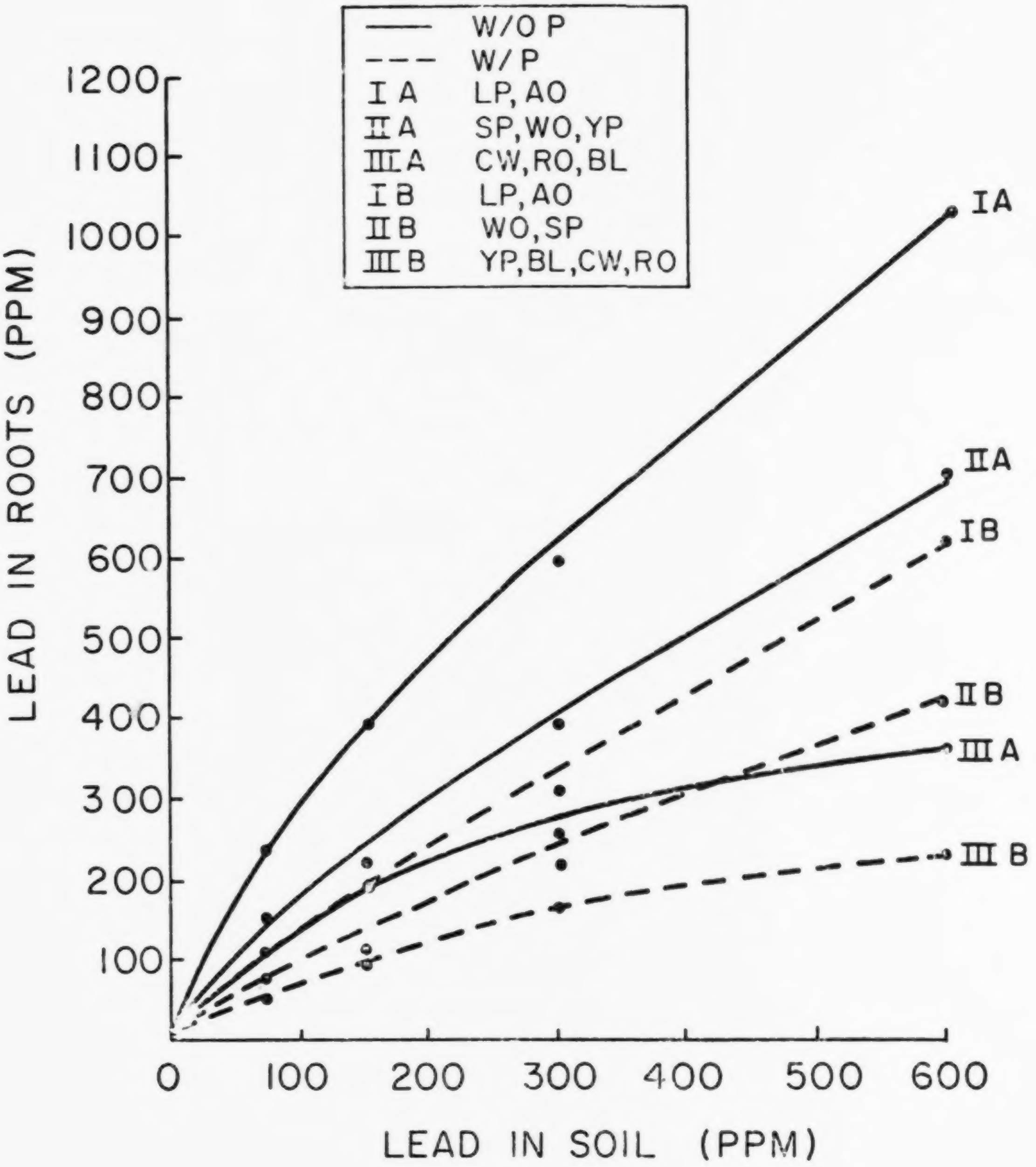

Figure 56. Root Lead Concentrations Derived from a Soil Source

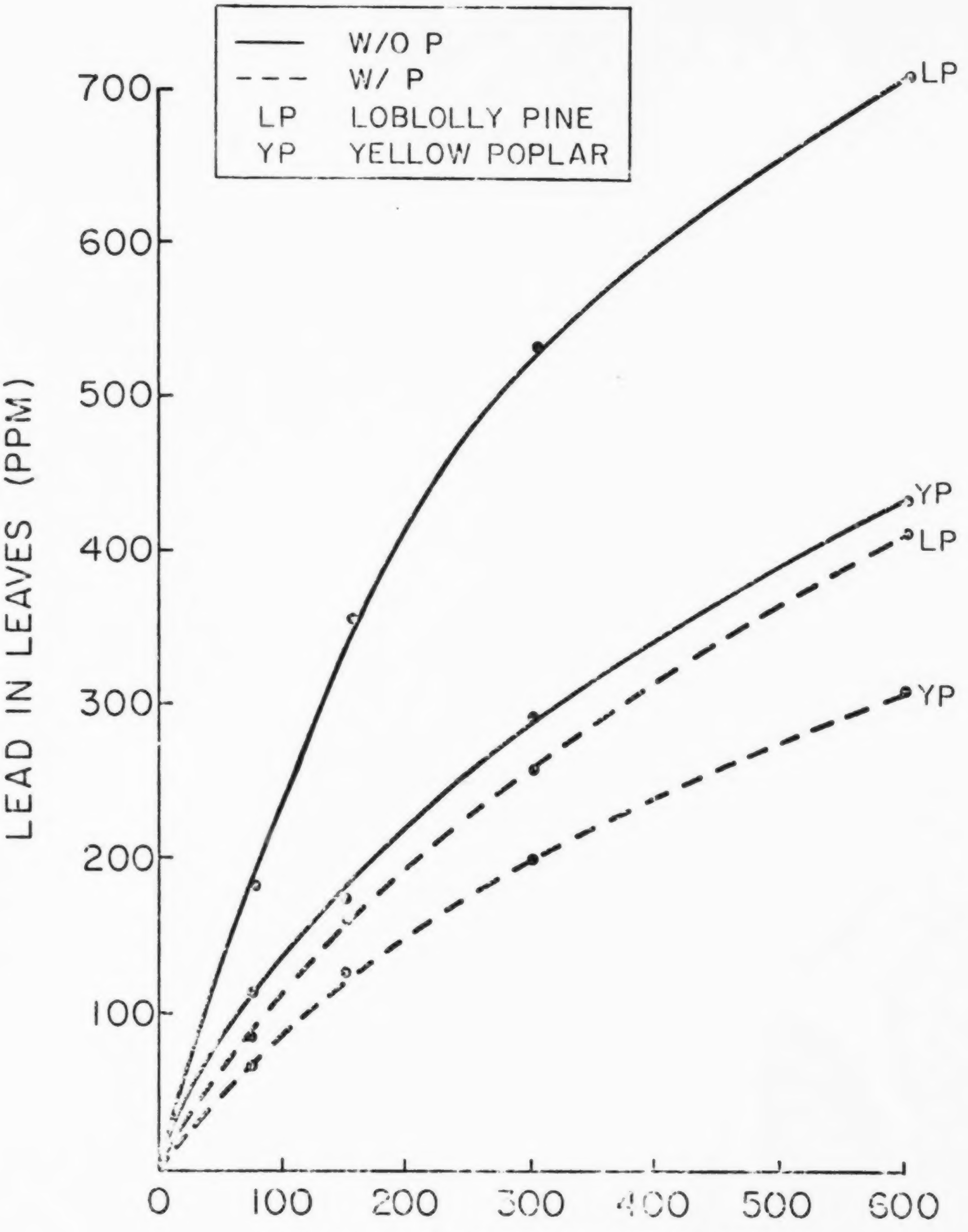

Figure 57. Leaf Lead Concentrations Derived from a Nutrient Solution Source

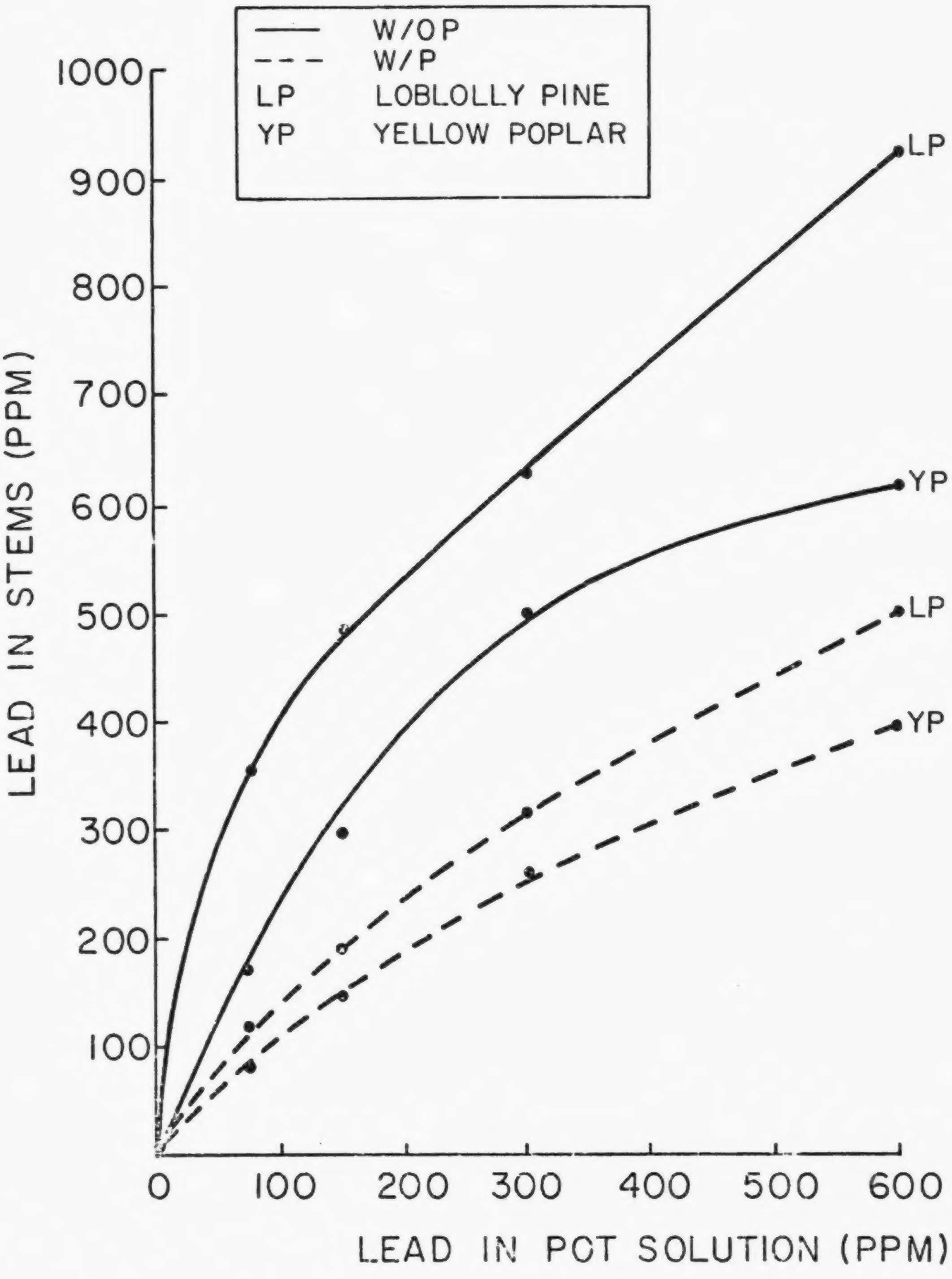

Figure 58. Stem Lead Concentrations Derived from a Nutrient Solution Source

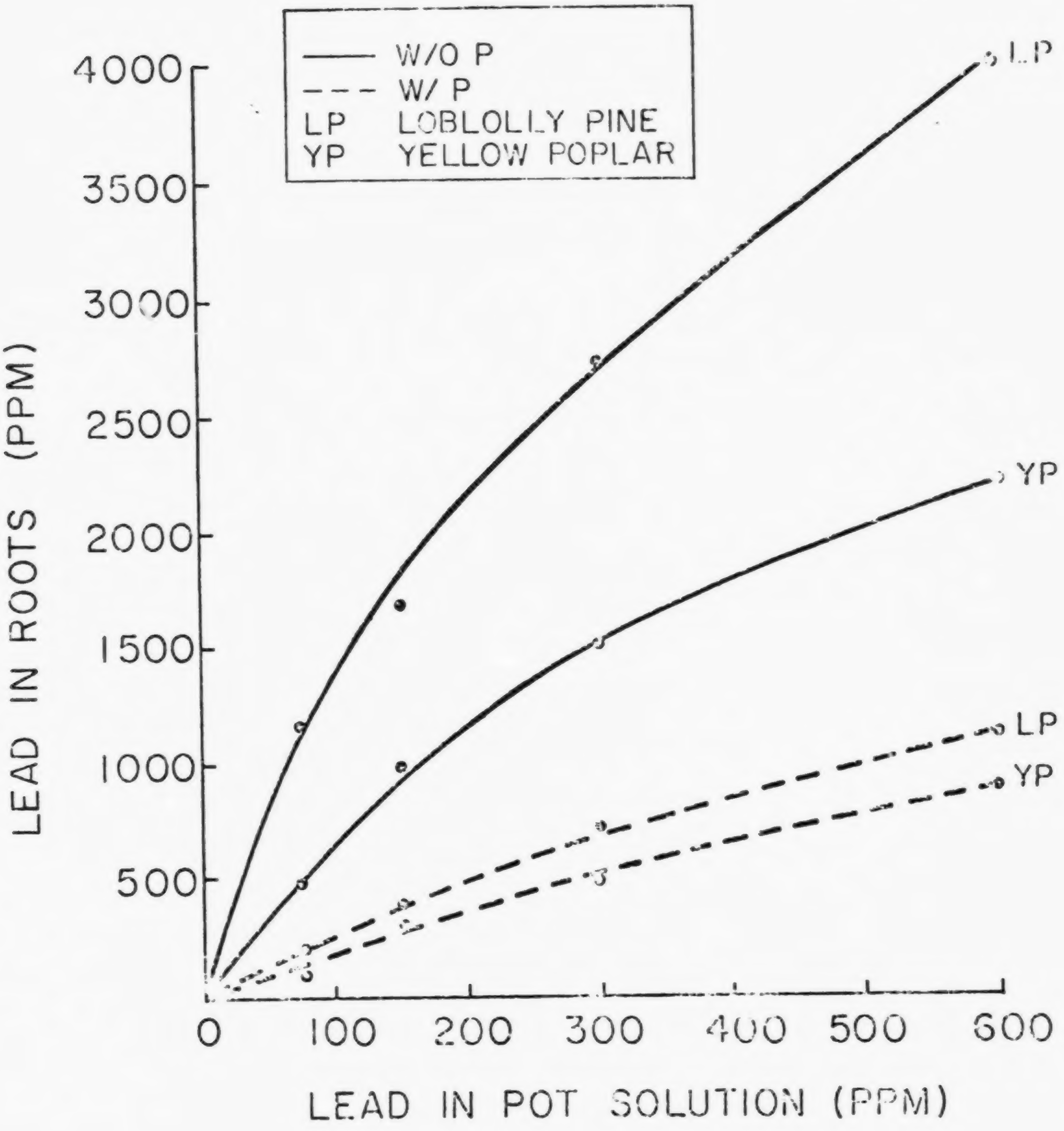

Figure 59. Root Lead Concentrations Derived from a Nutrient Solution Source

yellow poplar. These data indicate that the soil phosphate level is very important in controlling lead uptake by plants. Available soil phosphorus presumably ties up lead compounds in the form of lead phosphate precipitates which are insoluble and thus not available for plant uptake.

Most research involving lead uptake and translocation by plants has indicated that plants accumulate small amounts of lead from soil sources and that some lead taken up by the root systems is translocated to the aboveground portions of the plant but generally only small quantities. Most of this work, such as that of Motto _et al_., (1970), has been concerned with uptake of lead by crop species when very small amounts of soluble lead are added to the soil system. Under these conditons, only small amounts of lead accumulated in the plant. The rationale for using low soil lead concentrations in uptake studies is valid due to the fact that most agricultural soils away from concentrated lead sources have low lead levels. However, ultimately soil lead concentrations will rise, and we must predict what will happen at higher concentrations. In this present study, statistically significant levels of lead were found in the leaves, roots, and stems of eight tree species when grown in soil containing 75 to 600 ppm lead. Soil lead concentration significantly affected uptake with higher uptakes being associated with higher soil levels. The fact that significant uptakes over one growing season did occur at these high levels gives cause for concern. We must particularly be concerned with the possible effects in natural food chains as environmental lead levels rise. These effects may be subtle and directly affect man but, nevertheless, the ultimate effects might be disastrous.

Most of the lead seems to accumulate in the root system with significantly lower levels in the stem and leaves. Although the leaves accumulated the lowest concentrations, there was still sufficient lead in this portion of the plant to warrant further study of food chains based on this source of food and possible effects on plant growth and metabolism.

Soil conditions, other than phosphorus level, had no significant effect on uptake in this study but further research on soil factors affecting the mobility and activity of lead in the soil system should be initiated. The level of available phosphorus in the soil had a highly significant effect on lead uptake by all species. High levels of soil phosphorus reduced uptake by approximately one-half. This may be a significant factor in future control of lead movement in the soil system.

c. Earthworms

(1) Background The ubiquity of earthworms in many soils of temperate and tropical regions and their relatively low position in the food chain has prompted this study of lead and cadmium absorption in earthworm tissue. In England it was estimated that earthworms ingest 50,000 to 90,000 kg/ha of soil (oven dry st.) per year, equivalent of a layer of 3 to 6 mm of soil at a bulk density of 1.4 g/cc (Barley, 1961). In the course of many years, a large amount of mixing of the soil solum takes place, and much of the soil will have passed through an earthworm's gut at least once.

Lead is a heavy metal of particular concern in the environment because of its widespread distribution and relatively high concentration in urban and roadside soils (Lagerserff and Specht, 1970). Cadmium which is known to be toxic to living tissue is also deposited in roadside soils and is frequently present in sewage sludges from industrialized municipalities. With utilization of sewage sludge as fertilizer, Cd and Pb, which are also present in sludge, are being distributed on large areas of land. One mode of entry of these heavy metals into the food chain is through absorption and concentration by earthworms and subsequent ingestion of earthworms by higher animals.

Robins, amphibians, moles, small snakes, and fish are known to have earthworms as part of their diets (Elliott, 1968). Fish and some amphibians are included in the diets of a variety of mammals including man. Thus heavy metals can move up the food chain after absorption by earthworms.

Characterization of materials associated with earthworms have included castings, some components of the earthworm, and some components of soil. The latter have been primarily in connection with earthworm behavior. For example, their aversion or preference for resideing in soils at certain pH levels or with chemical amendments. Castings have been analyzed for nitrogenous and soil aggregate-stabilizing components in particular (Barley, 1961; Laverack, 1963; Parle, 1963). Evidence for stimulation of nitrogen mineralization by earthworms is the elevated nitrogen content of their castings. Polysaccharides are the materials primarily repsonsible for the enhanced aggregate stability of soil in castings. Reports of analyses for metallic components of earthworm tissue or castings are rare.

Calcium nutrition in earthworms has been studied in connection with the calciferous glands, and Ee in connection with Fe metabolism (Delkeskamp, 1964; Laverack, 1963). Reports of other heavy metals in tissue or castings have not been found.

(2) Procedures. Earthworm absorption of Pb and Cd from soil was studied in a greenhouse experiment. Drummer silty clay loam surface soil, 0-15 cm depth, which had been amended with lead acetate at the rate of 1600 kg/ha lead 2 years prior to collection was placed in 5 metal containers (30 x 21 x 6 cm) (Baumhardt and Welch, 1972). Similarly a silt loam, 0-15 cm depth, which had been amended with 0, 55, and 110 cm of liquid digested sludge (Calument Treatment Plant, Metropolitan Sanitary District of Greater Chicago) was prepared with 5 containers of each treatment. All containers were lined with polyethylene sheeting. Flanagan silt loam surface soil from

the University of Illinois, Urbana-Champaign farm, was placed in five 10.5 cm diameter clay pots to a 3 to 4 cm depth. Ground, dried corn plant tissue (3.0 g) was placed on the soil layer. The corn had been grown hydroponically in the presence of lead and contained 4650 ppm Pb oven dry wt. The corn tissue was covered with a thin layer of soil and 12 earthworms (*Eisenia rosea* Sav.) were placed in each pot. Earthworms were collected from fields on the university farm. Forty to fifty earthworms of the same species were placed in each of the other containers. Peat was added to the control and lead acetate-amended soils to provide organic matter for the earthworms. The containers were covered with perforated aluminum foil to prevent earthworms from crawling out and to reduce radiant heating. Soil moisture was maintained by watering with deionized water.

References regarding chemical analysis of earthworm tissue are rare. One report on nutrient composition of earthworms lists compound classes, but gives no elemental analysis (French, Liscinsky, and Miller, 1957). Because analytical chemical methods for earthworm tissue have not been widely reported, details are included here. Soil was tipped out of the containers and 2 to 4 earthworms were carefully removed from each of the 5 replicated treatment containers at each sampling. Thus replicate samples of several individual earthworms each were prepared. Earthworms were rinsed with deionized water and placed in containers of moist quartz sand. After 24 hours, they were recovered and placed, alive, in plastic vials and frozen. Visual inspection revealed that dark soil materials in the alimentary tract of the earthworms had been replaced by sand after 24 hours. The frozen earthworms were then freeze-dried. Subsequently they were placed in Vycor cruicibles and weighed prior to dry ashing at 500°C. The ash was weighed and dissolved in 10 ml of 1 N HCl. Lead and cadmium concentrations were determined by atomic absorption spectrophotometry. The difference between ashed weight and freeze-dried weight was considered dry tissue weight for purposes of calculating concentrations. This appeared to be most accurate because of the variable amounts of sand ingested and retained by earthworms.

(3) Results and Discussion. Liquid digested sewage sludge amendments increased lead and cadmium concentrations in soil over the control (Table 34). Although the control soil of the sludge group and the soil amended with plant tissue came from different areas on the university farm, lead and cadmium concentrations were similar.

At the 2-day sampling, lead concentration in earthworms from the control and maximum sludge-amended soils was the same (Figure 60A). After 21 days, earthworms in sludge-amended soils showed increased Pb concentrations. Lead concentrations for all treatments are means for 2 to 6 replicates.

Earthworm survival was poor after placement in sludge-amended soils. Some died with no visible morphological changes. Others developed skin lesions and welts. The cause of these conditions is unknown, but an organic etiological agent is more likely than an inorganic agent. Some organic compounds are known to be irritating to earthworm skin (Gersch, 1954; Lagrange, 1946). These reactions were not observed in any sludge-free soils. Worms which survived the initial 1 to 3 days remained active for another 2 to 3 weeks. Because of the limited number of earthworms available, and high replacement rate, long-term experiments with the sludge-amended soils were not attempted. Apart from obvious physiological damage, the number of earthworms escaping from sludge-amended soil indicated that they were not content in it. Earthworms in other soils settled at the bottom of the container and remained there.

Table 34 .

Lead and Cd concentrations in silt loam soils used experimentally.

Amendments	Pb* ppm	Cd* ppm
0 sludge	32	2
55 cm sludge	97	18
110 cm sludge	142	30
1600 kg/ha Pb	624	2
Pb-containing plant tissue	35**	2

*By atomic absorption spectrophotometry on 1 N HCl soil extracts (13).

**Plus 13.95 mg Pb in plant tissue.

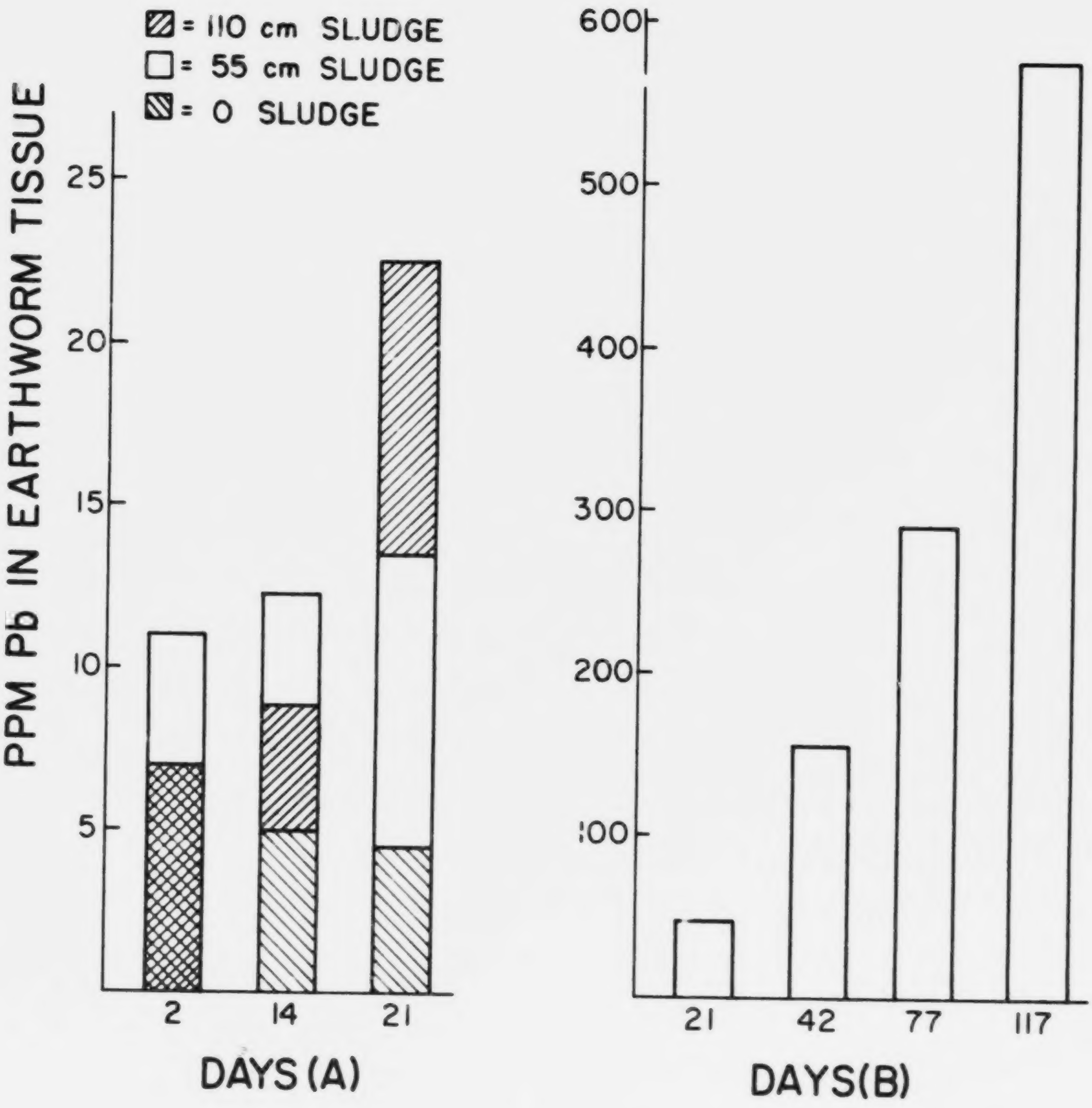

Figure 60. Lead concentration in earthworm tissue of earthworms living in sludge-amended soil (A) and Pb acetate-amended soil (B) for the number of days indicated.

Lead concentration was highest in lead acetate-amended soil and in the associated earthworm tissue (Figure 60B). No visible adverse effects from the lead were observed. Each sample reported was the mean of 3 to 6 replicates. One individual tissue sample (composed of several individual earthworms) contained the maximum lead concentrations of 981 ppm. Absorption of lead in this degree from soil amended with a lead salt appears significant relative to environmental conditions near roadsides and in urban areas. Lead concentrations are reported in excess of 500 ppm in surface layers of roadside soils (Lagerwerff and Specht, 1970).

Heterogeneity of the soil-corn plant tissue mixture and small earthworm populations limited the reliability of those lead absorption data. Concentrations as high as 86 ppm were found in single samples, while samples collected simultaneously were as low as 13 ppm.

Cadmium absorption by earthworms did not change appreciably with time or treatment. The only cadmium addition which was made to soil was as a component of sewage sludge. After two days exposure to sludge-amended soil, the range in Cd concentration in replicate earthworm samples was 2.7 to 10.2 ppm. After 21 days, replicates ranged from 1.8 to 15.0 ppm. The range in other soils was from 2.3 to 25.3 ppm.

D. Personnel

Aerosol Lead Particles

K. Boyer
Dr. H. A. Laitinen

Movement of Lead in Soil-Water System

Dr. O. C. Braids
F. J. Drone
Dr. R. Gadde
Dr. H. A. Laitinen
J. E. Bittell

Movement of Lead (and Cadmium) in the Soil-Water-Organismal System

J. E. Bittell
R. R. Root
Dr. D. E. Koeppe
Dr. R. J. Miller
Dr. R. Gadde
Dr. H. A. Laitinen
Dr. G. L. Rolfe
Dr. O. C. Braids

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VI. PLANT PROCESSES

INTRODUCTION

Lead effects on vital plant processes directly related to growth and survival were studied at the levels of plant organization. First was the study of gas exchange properties,i.e., photosynthesis, respiration, and transpiration of whole intact plants. The next level was a subcellular study of photosynthesis and respiration respectively on isolated chloroplasts and isolated mitochondria. These subcellular studies dealt with mechanisms of the effect of lead on plants.

A. Effects of Lead and Cadmium on Whole Plant Photosynthesis, Respiration and Transpiration of Dominant Species in the Watershed Ecosystem

The studies are concerned with effects of lead, supplied to plants as $PbCl_2$ in nutrient solution, on gas exchange properties of maize which is the most dominant plant in the watershed ecosystem. Experiments were made using young (ca. 25 day old) plants and under optimum light, temperature, and moisture conditions. Future experiments will include soybean, another crop plant, and a few weedy and tree species common in the watershed. Furthermore, studies will be made under stress conditions, e.g., low water potentials and high temperature. The effects of lead compounds as topical materials on gas exchange properties will be conducted during summer and continued during fall. This is contingent upon the completion of development and construction of an aerosol chamber in which size particle could be precisely controlled.

Zea mays L. plants were grown from commercial seed variety GSC 7x27 NLREA obtained from Gildersleeve Seed Co., Champaign. Seeds were soaked in distilled water and planted in plastic flats containing vermiculite. The flats, each with six seeds, were incubated in a growth chamber under 1800 ft-c. and 16 hr. photoperiod. Daytime temperature was 27°C and nighttime temperature was 17°C. The plants were watered with Hoagland solution on the 7th day of growth and on the 11th day lead as $PbCl_2$ was added in the following concentrations: 0, 50, 100, 150, 300, 600, 1000, 2000, 4000 and 8000 ppm. The plants were allowed to grow until the leaves were large enough to be used for gas analysis at approximately day 25 after germination. Attached single leaves were inserted through a side port in the assimilation chamber and were held horizontally by a thread grid inside the chamber. The air stream circulating between the assimilation chamber and an infrared gas analyzer was saturated with moisture at the beginning of each experiment. Relative humidity was controlled at 70 ± 2% wind speed at 1.0 m sec^{-1}, ambient temperature at 25 ± 0.5°C and carbon dioxide concentration between 300 and 310 ppm. Maximum light intensity at the plant level was ca. 5000 ft-c. (0.50 cal cm^{-2} min^{-1}), generated by seven 300 W spotlights partially immersed in a water bath. The light beam was passed through Corning infrared filters to further remove infrared radiation and through a neutral density filter to reduce intensity. This procedure insured that leaf temperatures (measured with a copper constantan thermocouple) and ambient temperature in the chamber were very close (±0.6°C). Apparent photosynthesis as net CO_2 uptake was measured after a steady rate of gas exchange between the enclosed leaf and the circulating air stream. Transpiration was measured simultaneously by a method which depends on using the plant as humidifier and measuring change in ambient relative humidity in the air stream. After several similar readings of photosynthesis and transpiration, dark respiration was measured as CO_2 release after a new steady state of gas exchange between the leaf and the circulating air had been achieved. The leaves were then removed, their area measured and chlorophyll, extracted from another comparable leaf on the plant by acetone, and total chlorphyll a + b, were determined spectrophotometrically. The leaves were oven dried to constant weight at 85°C and then ashed in a muffle furnace at 400°C. Lead content of the leaves was determined and is expressed in ppm dry wt.

Preliminary data indicate that roughly 10% of the lead supplied to the soil is accumulated in the corn leaves. The data also show that photosynthesis decreases sharply as Pb concentration increases to 1000 ppm (Figure 61). A slight increase in photosynthesis was observed at 2000 ppm Pb and again a slight decrease at 4000 ppm. Plants grown in vermiculite with 8000 ppm lead were extremely weak, small, and most of their leaf blades were necrotic. Net photosynthesis in these leaves was essentially zero.

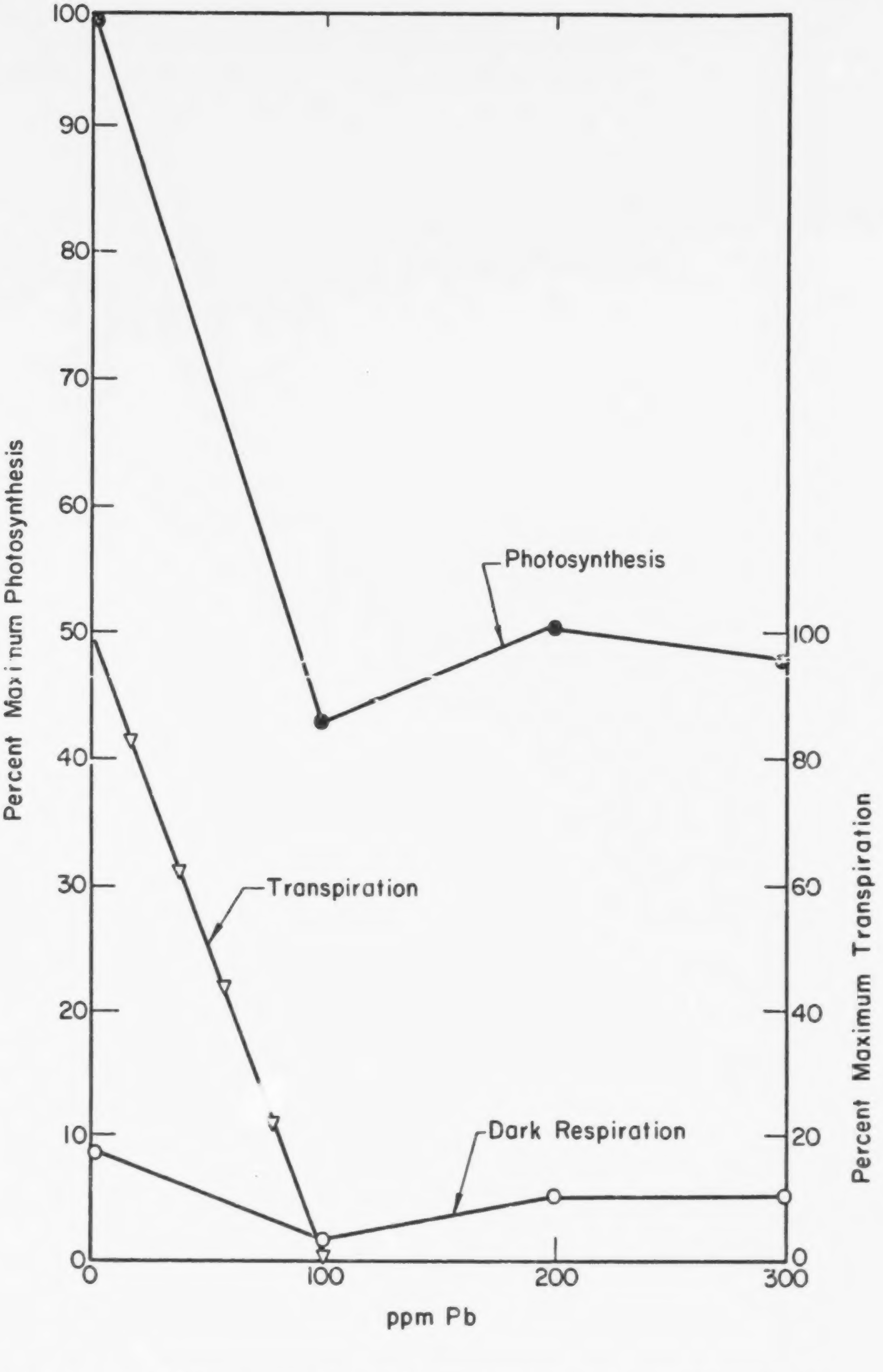

Figure 61. Net photosynthesis, dark respiration and transpiration of maize plants grown in vermiculite containing various concentrations of $PbCl_2$.

Dark respiration which was much lower than photosynthesis (1/12) followed similar trends except at 1000 ppm lead where it was negligible. However, at 2000 and 4000 ppm lead respiration was about 50% of maximum. No measurements of dark respiration at 8000 ppm lead were obtained.

Transpiration declined to essentially zero at 1000 ppm lead and was not measurable at lead concentrations above 1000 ppm (Figure 62).

Morphologically, plants grown in vermiculite with 8000 ppm lead were severely stunted. Their leaves in addition to being small were tightly rolled and generally wilted. They were pale green with white and purplish streakings and their edges were necrotic. Plants grown in 4000 ppm lead exhibited similar trends but to a much lesser degree. The leaves averaged 32 cm in height while the controls (0 ppm) averaged 43 cm. Morphological differences between plants grown in 2000 and 1000 ppm lead and controls were observable and generally a very gradual gradient in size and greenness was detectable.

At the present, these experiments are being repeated on greenhouse-grown corn plants and the effects at concentrations between 0 and 1000 ppm will be investigated in more detail and will be replicated several times. Plants of other species (soybeans, wheat and black oak) will be studied in a similar fashion during June, July and August. Work is progressing on the development of an aerosol chamber which will be used to study the effect of lead compounds (as topical materials on leaves of the above mentioned plants on their gas exchange properties).

The effect of cadmium on whole plants gas exchange properties is being investigated. Sunflower plants grown from seed in growth chambers under 27°C day and 20°C night temperature, 1800 ft-c. and 15-hour photoperiod were used in the experiments. Single leaves were enclosed in the assimilation chamber and their petioles were cut under degassed water.

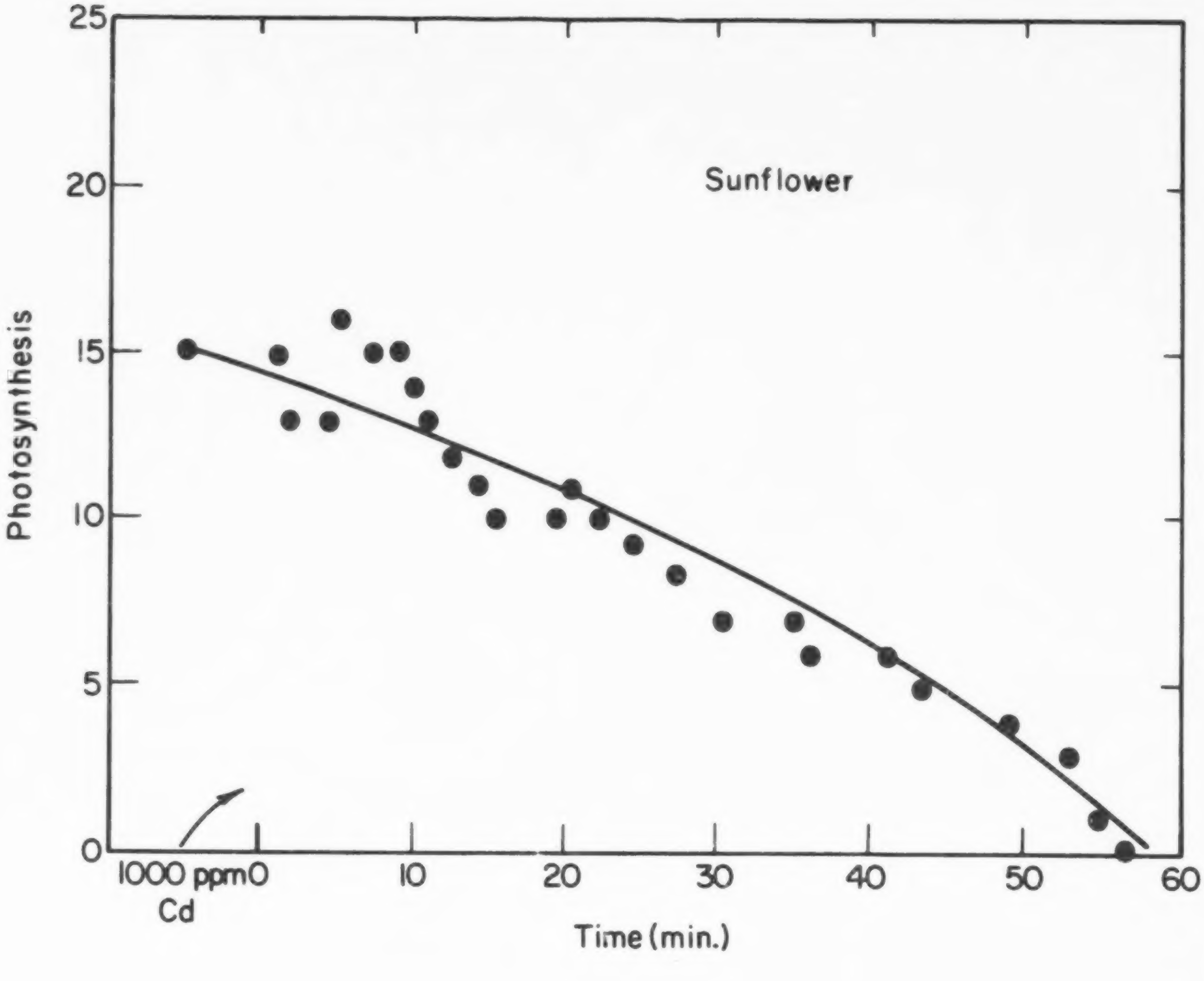

Figure 62. Effect of $CdCl_2$ on photosynthesis and transpiration of sunflower detached single leaves. Cadmium chloride was supplied in the water in which leaf petioles were immersed.

Rates of photosynthesis and transpiration were monitored simultaneously for about an hour to insure equilibration of gas exchange between the leaf and its surrounding air. Various concentrations of Cd as $CdCl_2$ solution were added to the water in which the tip of the petiole was immersed and photosynthesis and transpiration were continuously measured until they were reduced to essentially zero. Time course responses for several Cd concentrations are presented in Figures 63 and 64 . The leaves were removed from the chamber, oven dried at 85°C to constant weight and their Cd content determined.

Preliminary results indicate that Cd has drastic effects on gas exchange properties of single detached sunflower leaves. Generally, a rapid increase in transpiration began about a minute or two after the introduction of Cd. A peak in transpiration was reached about 5 min. after Cd introduction and a very sharp decline in rate followed (Figure 64). After about 12-15 min. transpiration became undetectable. Initial response of photosynthesis to Cd addition is somewhat slower than that of transpiration for no detectable effects of Cd on photosynthesis occurred during the first 5 min. However, a rapid decline occurred after that and the photosynthesis was undetectable after 10 min.

To investigate whether or not the decline in gas exchange rates between leaves and air is due to rapid stomatal closure only, carbon dioxide concentration around the leaves was increased from 300 to 600 ppm and photosynthesis monitored under these conditions. Since photosynthesis was not detectable at this high concentration it is concluded that the effects are not due to stomatal closure only. Apparently there was major inhibition of the enzymatic reactions of the photosynthesis system itself.

A monodisperse aerosol generator is desirable for studies which focus on the interaction of aerosols with plants and animals. This project has as

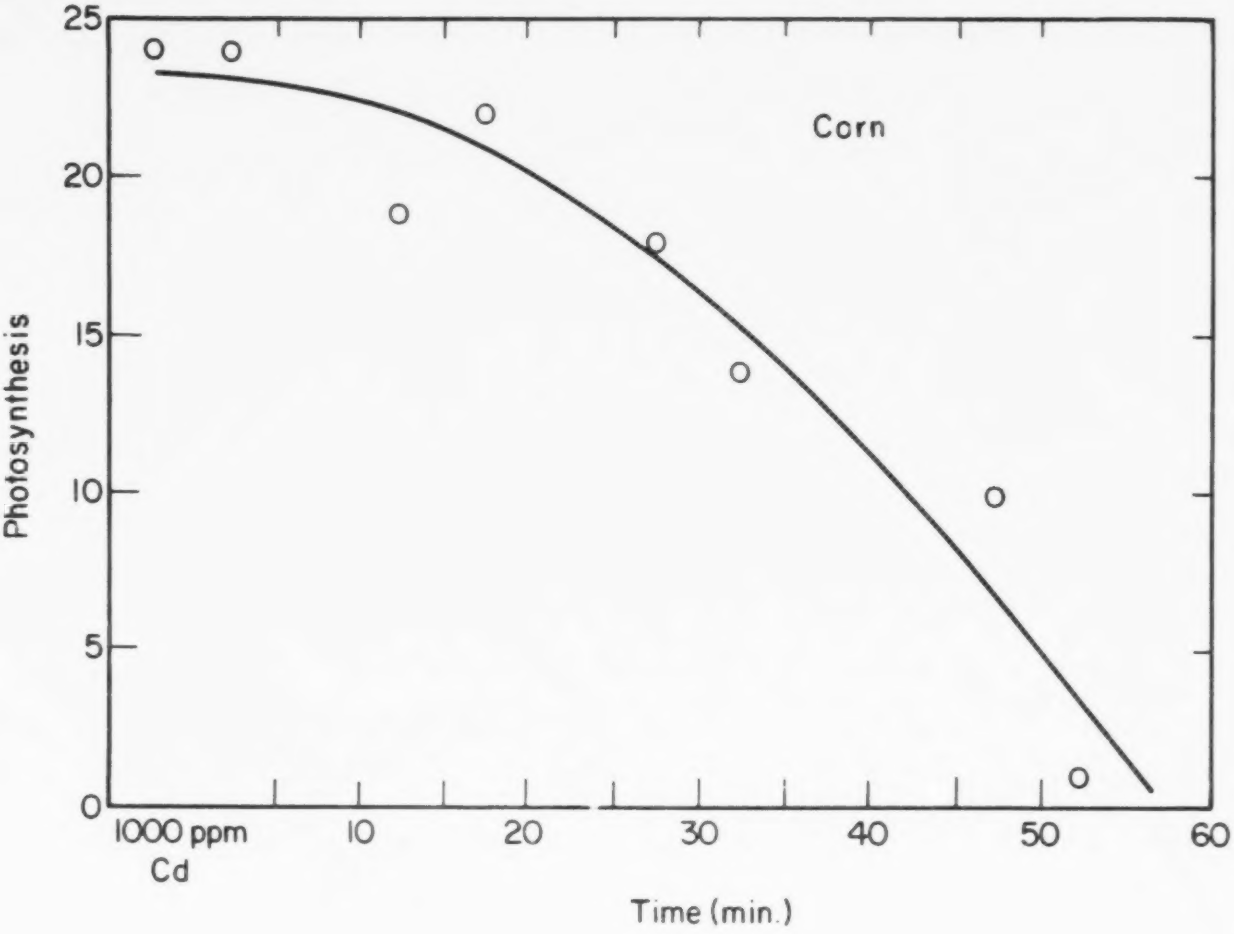

Figure 63. Effect of $CdCl_2$ on photosynthesis and transpiration of maize detached single leaves. Cadmium chloride was supplied in the water in which leaf petioles were immersed.

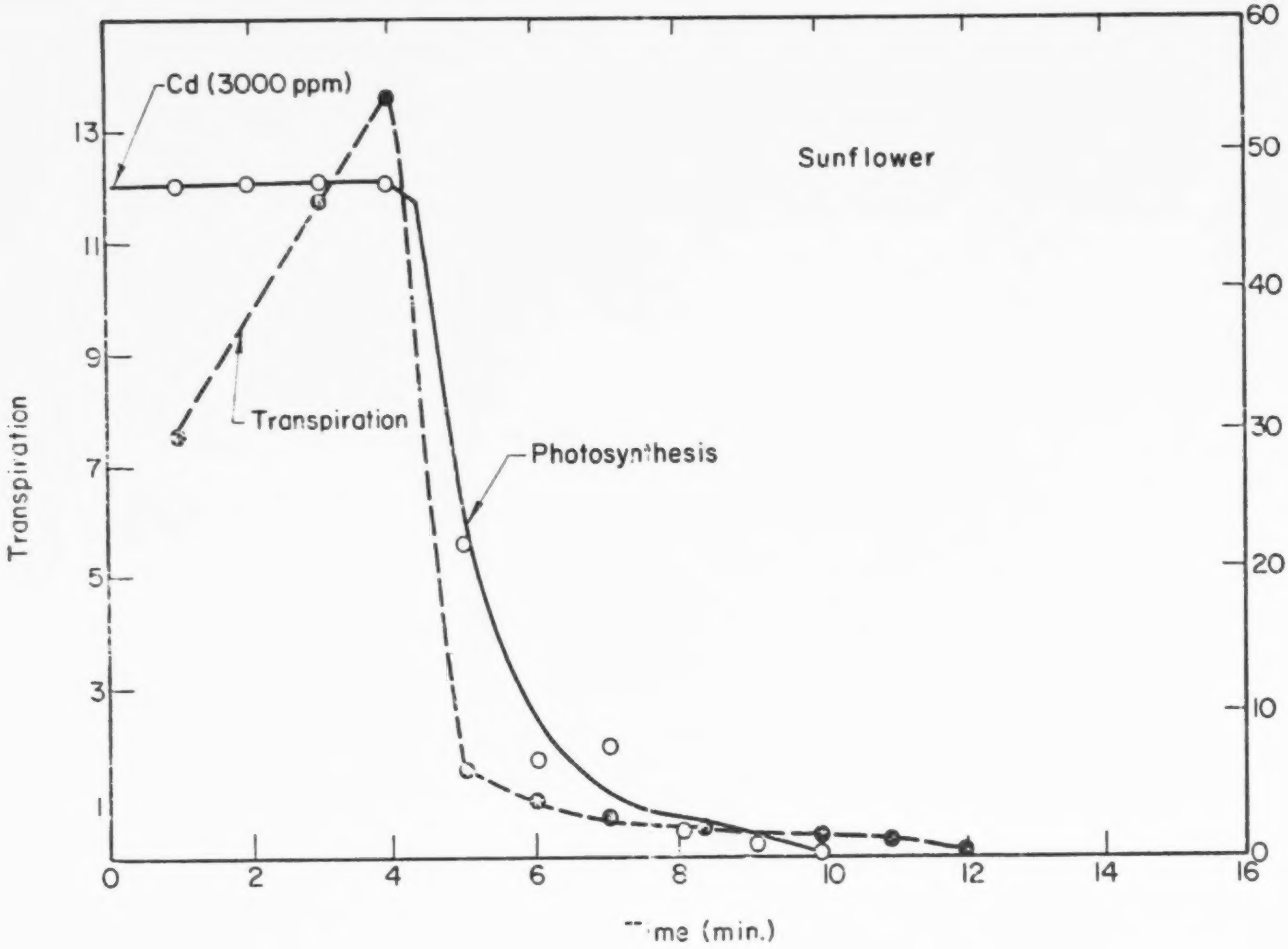

Figure 64. Effect of $CdCl_2$ at high concentrations on photosynthesis and transpiration of detached single leaves of sunflower. Cadmium chloride was supplied in water in which the petiole was immersed.

its objective the generation of a monodisperse aerosol of preselected size, concentration and composition.

The general methods of aerosol generation which were considered included (1) the dispersion of a pre-classified powder, (2) vapor condensation and (3) atomization of a liquid-liquid suspension or liquid solution. For our particular application, methods (1) and (2) were rejected in favor of atomization-type generators. Atomization mechanisms which were considered for aerosol generation include ultrasonic sound, nozzles and spinning disks. At the present time, an ultrasonic generator is under test.

The principle of operation of the present apparatus involves the excitation of a piezoelectric material with a high frequency ultrasonic signal. The ensuing mechanical vibration is used to disturb a liquid jet as it passes through an aperature (in the 10μ size range). This action renders the jet unstable and causes the liquid to assume a more stable configuration-monodisperse spheres. To avoid nearly immediate coalescence of the aerosol, it is passed instantaneously into a high velocity nozzle which causes it to disperse.

While complete characterization studies are still in progress, it is believed that the present design provides a unique, compact, flexible method for the generation of a monodisperse aerosol.

B. Effect of Lead Salts on Photosynthetic Reactions in Isolated Chloroplasts

Photosynthesis in green plants requires the cooperation of two light reactions, sensitized by two pigment systems (PS I and PS II). A model involving the cooperative interaction of two light reactions acting in series is presented in Figure 65. A series model postulates that the first light reaction, arbitrarily called photoreaction II, forms a strong oxidant (Z^+)

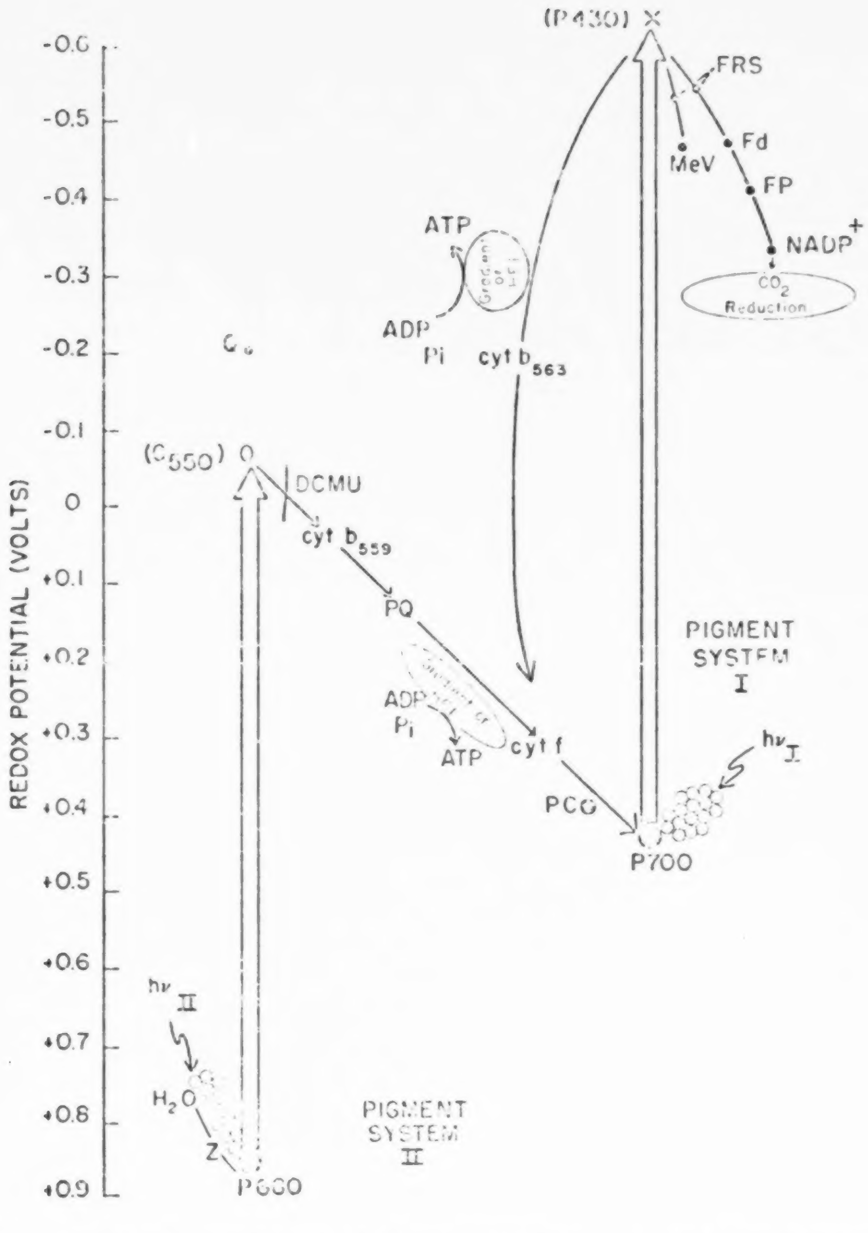
REDOX POTENTIAL (VOLTS)
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
0
+0.1
+0.2
+0.3
+0.4
+0.5
+0.6
+0.7
+0.8
+0.9
(P430) X
FRS
MeV
Fd
FP
NADP$^+$
CO$_2$ Reduction
ATP
ADP
Pi
cyt b$_{563}$
(C$_{550}$) Q
DCMU
cyt b$_{559}$
PQ
ADP
P$_i$
ATP
cyt f
PC
P700
PIGMENT SYSTEM I
hν_{I}
hν_{II}
H$_2$O
Z
P680
PIGMENT SYSTEM II

Figure 65. Two light reactions in photosynthesis. Z, primary electron donor of light reaction II; P680, energy trap of pigment system II; Q, primary electron acceptor of system II - probably equivalent to C550; PQ, plastoquinone; PC, plastocyanin; P700, energy trap of pigment system I; X, primary electron acceptor of system I - equivalent to P430; Fd, ferredoxin; Fp, Flavoprotein; NADP$^+$, Nicotinamide adenine dinucleotide phosphate; FRS, Ferredoxin reducing substance; ATP, adenosine triphosphate; HEI, high energy intermediate; MeV, methyl viologen.

and a weak reductant (Q^-). A second reaction, photoreaction I, forms a weak oxidant ($P700^+$) and a strong reductant (X^-). The two systems are connected by a chain of electron carriers through which the reduced Q passes electrons (or H atoms) to the oxidized P700. Plastoquinone (PQ), Cytochrome (Cyt)b_3, Cyt f, and Plastocyanin (PC) are implicated as successive members of this chain. Adenosine triphosphate (ATP) is generated through phosphorylation that is coupled with electron transfer. Two types, called non-cyclic and cyclic photophosphorylation (PP), are distinguished. In the non-cyclic PP, the formation of ATP is coupled with electron flow from Q^-(E.∿0.0v) to $P700^+$(E.∿0.4v), and finally leading to nicotinamide adenine dinucleotide phosphate ($NADP^+$) reduction and oxygen evolution. Carbon dioxide fixation in plants occurs via either the Calvin-Benson Pentose Cycle in C_3 plants or Hatch and Slack C_4-dicarboxylic cycle in C_4 plants (Hatch & Slack, 1970). Maize which is utilized for this study is a C_4 plant and has two different types of chloroplasts contained in mesophyll and bundle sheath cells. The chloroplasts differ from each other not only in spatial distribution and ultrastructural characteristics but also in activities of PS II, and in the enzymes involved in carbon dioxide fixation.

Our proposed research problem was to study the effect of heavy metals and their mode of action on the light reactions of photosynthesis in isolated chloroplasts of maize. Two approaches were proposed to introduce the heavy metals to chloroplasts: (1) In vitro approach which involves the addition of salts of heavy metals to the reaction mixture and comparing the rates of the reaction in the presence of lead salts with the rates of untreated chloroplasts in order to determine the kind of effect these heavy metals have on chloroplasts. (2) Feeding heavy metals to plants by growing them in vermiculite and watering with Hoagland solution containing salts of the heavy metals, the effects of which on photosynthesis are to be studied.

We started our research by using the first approach and we chose to study first the effect of the heavy metal lead on the light reactions of photosynthesis in isolated chloroplasts. We began experiments to test the effect of lead salts on PS I and PS II in isolated chloroplasts of maize.

To study the effect of various concentrations of lead salts such as $PbCl_2$ and $Pb(NO_3)_2$ on PS I, we measured the rate of $NADP^+$ photoreduction in 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU) treated chloroplasts with reduced 2-6, dichlorophenol indophenol ($DCPIPH_2$) as electron donor to PS I. Rates of $NADP^+$ photoreduction were determined by measuring the increase in absorbance at 340 nm upon illumination of samples in a Cary-14 spectrophotometer equipped with a side-light exciting beam attachment. A crude preparation of the enzyme, Photosynthetic phosphopyridine nucleotide reductase (PPNR), required for $NADP^+$ photoreduction in isolated chloroplasts was prepared according to the method of San Pietro and Lang (1958). This method requires the use of 0.5M tris-HCl pH 8.0 buffer as a suspending medium for this enzyme. When we started to test the effect of lead on $NADP^+$ photoreduction using this enzyme preparation, we were faced with some difficulties. The lead chloride interacted with some components of the reaction mixture used. For example, we found out that 2.4 mM (and also lower concentrations) of lead chloride interacted with tris (Sigma Chemical Co. and Harleco Co.) and caused precipitation. Since the enzyme was suspended in 0.5M tris, we had to free it of tris and suspend it in another buffer which did not cause precipitation of lead chloride. We tested several buffers and we found that Tricine (Calbiochem Co.) buffer did not cause precipitation. We prepared another batch of the enzyme (PPNR) which was suspended finally in 0.05M Tricine buffer pH 8.0. We then tested to see if lead chloride still caused precipitation of the PPNR; indeed it did. This indicated to us that some component of PPNR must be complexing with lead chloride, and thus causing precipitation even when it is in Tricine. This would suggest that lead chloride could precipitate this important enzyme and cause a reduction of photosynthesis of plants. Now we are trying to investigate this point to be sure that this complexing of lead with the PPNR is occurring in vivo. We are also trying to overcome some of the difficulties, arising from complexing of lead and the enzyme, to be able to measure the effect of lead on $NADP^+$ photoreduction in isolated chloroplasts.

We also tested the effect of lead chloride on PS II, by measuring the photoreduction of DCPIP in isolated chloroplasts at saturating light intensities. Lead chloride seemed to react with this dye and caused slight bleaching; however, with appropriate controls to correct for this, we were able to measure PS II activity in the presence of various concentrations of lead chloride. Our results (Figure 66) show maximum inhibition of about 60% of DCPIP photoreduction at 400μM $PbCl_2$, while at higher concentrations slight recovery of inhibition was observed. This point needs to be further confirmed. However, if this is true, it indicates that the effect of lead on PS II is complex and is not only inhibitory. This implies that lead chloride at higher concentration than 400μM can feed electrons to PS II as it has been shown for NH_2OH in algae (Bennoun & Joliot, 1969; Mohanty, et. al., 1971) and in isolated chloroplasts (Bennoun & Joliot, 1969; Iazwz, et. al., 1969).

We also tested the effect of lead chloride (50μM, 500μM and 750μM) on the time course of Chlorophyll (Chl) fluorescence in isolated maize chloroplasts. Dark adapted chloroplast samples, upon illumination with strong blue light, show instantaneous rise in fluorescence intensity to an initial level F_o which reflects fluorescence emitted by the bulk pigments of PS I and PS II; thereafter fluorescence level rises slowly and biphasically

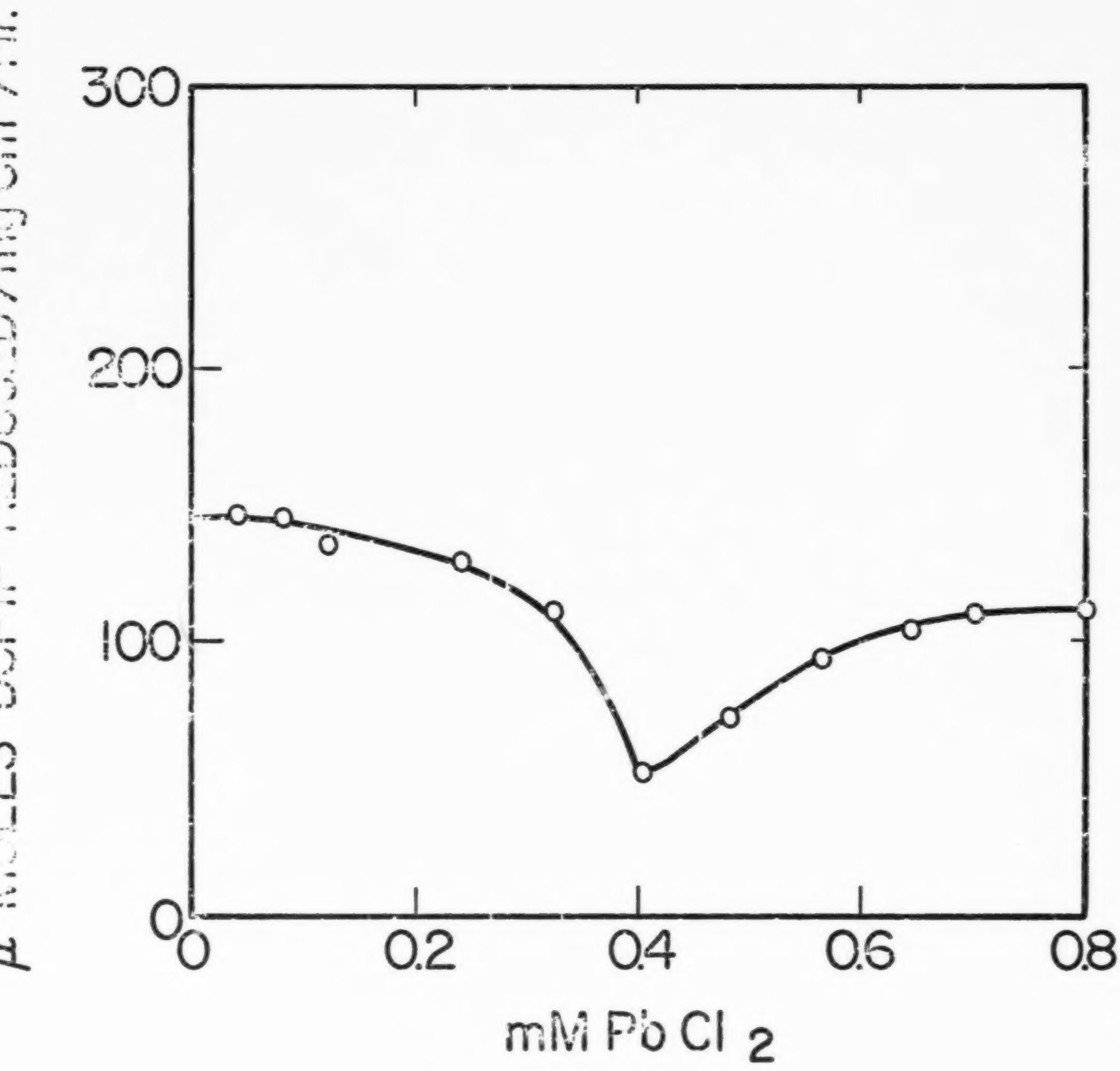

Figure 66. Rates of DCPIP photoreduction in isolated chloroplasts as a function of $PbCl_2$ concentration. Reaction mixture containing 0.05 M Tricine buffer pH 8.0; 3.5×10^{-5}M DCPIP; 0.01 M NaCl; and chloroplasts (20 μg/3 ml reaction mixture). Intensity, 2×10^5 ergs/cm^2 sec. Time of illuminiation, 45 sec.

to F_∞. The fluorescence rise from F_o to F_∞ reflects the photoreduction of Q (the primary electron acceptor of PS II) and when this acceptor is oxidized the fluorescence intensity is quenched. (For further discussion of Chl fluorescence transients see Govindjee & Papageorgiou, 1971). Studies of fluorescence transients, therefore, could be used as an indicator of the photosynthetic electron flow and the primary photochemical events in system II. We measured the effect of various concentrations of $PbCl_2$ on the time course of fluorescence. Figure 67 is a plot of the relative yield of variable to constant fluorescence as a function of $PbCl_2$ concentration. It is clear from this curve that even low concentrations of lead (50 μM) are enough to cause about 40% inhibition of the variable fluorescence of Chl a which reflects PS II activity. At 750 μM $PbCl_2$ maximum inhibition of about 64% of variable fluorescence is observed. This result confirms the measurement of DCPIP photoreduction in isolated chloroplasts, i.e., lead chloride is causing an inhibition of PS II activity.

We have also measured the emission spectra of Chl a at 77°K in order to detect any effect of $PbCl_2$ on the conformational status of membranes in chloroplasts which in turn results in changes in the rate of spill-over of light energy from PS II to PS I. Murata (1969), Homann (1969) and Mohanty (1971) in our laboratory have shown that divalent cations such as Mg^{++} could control the spill-over of energy absorbed by PS II to PS I. In the presence of 33μM $MgCl_2$, spill-over of energy from PS II to PS I is prevented and increase in system II fluorescence yield at room temperature is observed. This could be further confirmed by measuring emission spectra of Chl a fluorescence at 77°K. Pigments of PS I are weakly fluorescent at room temperature but they become highly fluorescent at 77°K with a band of peak maximum at 735 nm in isolated chloroplasts and at 720 nm in Chlorella. If lead is affecting photosynthesis by causing conformational changes of membranes

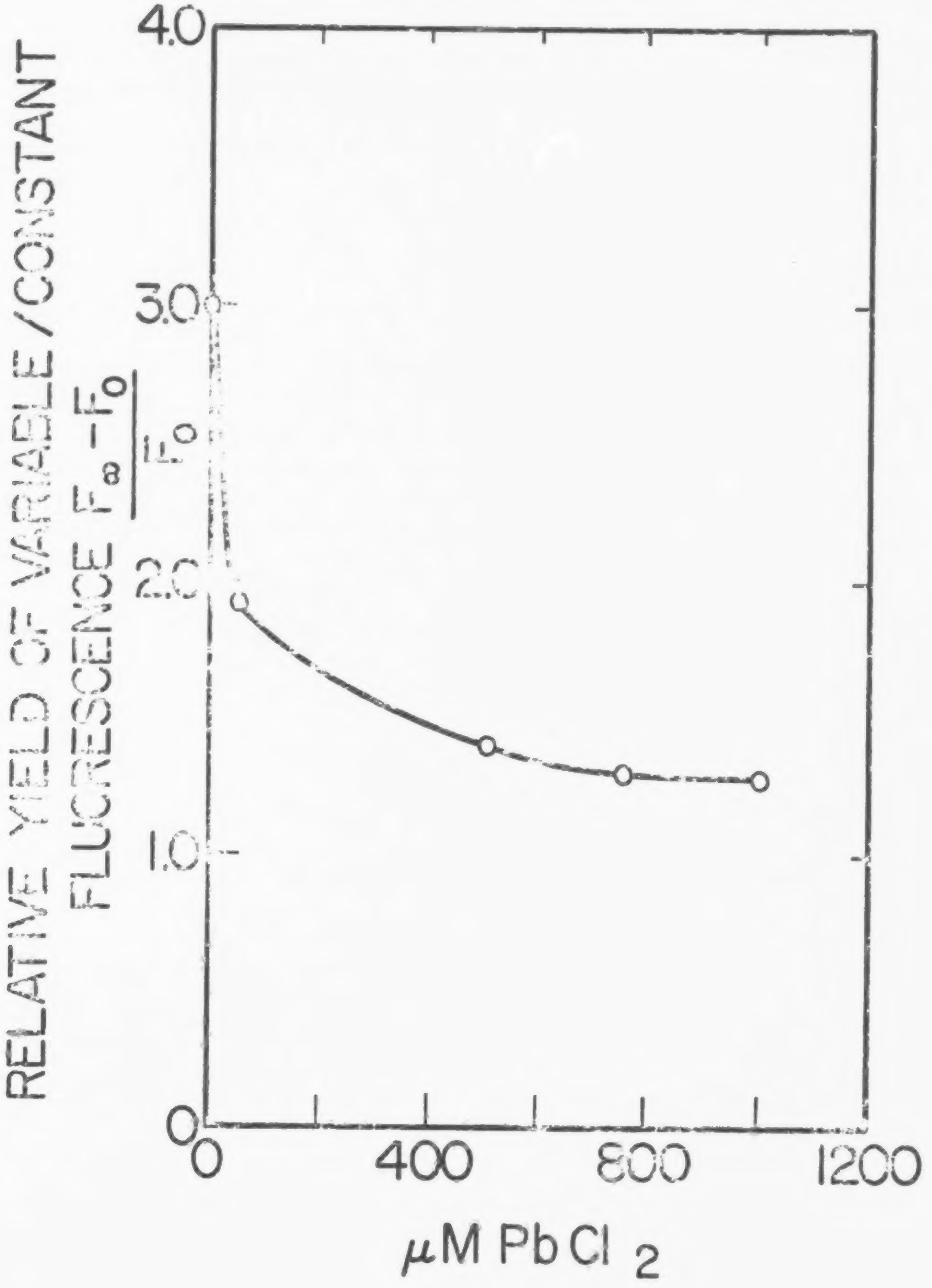

Figure 67. Relative yield of variable/constant fluorescence of maize chloroplast suspensions (5 μg [Chl]) as a function of $PbCl_2$ concentration. Wavelength of excitation, broad blue-green band with a peak maximum at 480 nm of 2×10^5 ergs/cm^2 sec. Measuring λ, 685 nm.

(thylakoids) of chloroplasts, we could detect it by comparing emission spectra of untreated and treated chloroplast suspensions at 77°K. Figure 68 presents such spectra. Normal chloroplasts, at 77°K, show a three-banded structure with peaks at 735 nm, 685 nm and 693 nm. (For further discussion see Govindjee, et. al., 1967.) F735 has been attributed to emission by pigments of PS I while F685 is emitted mainly from PS II. F 693 is attributed to fluorescence from the traps of PS II. Thus, a comparison of the ratio of F735/F685 should reflect the distribution of absorbed quanta between the two photosystems. This ratio (determined from Figure 68) is 3.9 in untreated chloroplasts compared to 2.5 for chloroplasts treated with 500 μM $PbCl_2$. Also the F693 to F685 is decreased in treated compared to normal chloroplasts. The effect of lead on decreasing F735/F685 is similar to the effect of Mg^{++} in isolated chloroplasts. However, the decrease in F693 relative to F685 is similar to the effect reported for NH_2OH (Bennoun & Joliot, 1969; Izawa, et al., 1969; Mohanty, et. al., 1971). Thus from this type of measurement it seems that lead is affecting maize chloroplasts in a way similar to both Mg^{++} and NH_2OH.

To further understand the effect of lead chloride on PS II activity, we measured the effect of various concentrations of lead chloride on delayed light emission (DLE) in chloroplasts. Assuming that energy available for DLE is provided by a back reaction of system II ($Z^+ Chla_2Q^- \rightarrow 2Chla_2Q$ + energy) (Clayton, 1967; Lavorel, 1969; Stacy, et. al., 1971) then this assay could provide further information about the mode of action of lead on PS II in chloroplasts. The slow DLE which we measure in our setup has been suggested by Jursinic and Govindjee (1971) to involve in addition to the back reaction of Z^+ with Q^-, a reducing entity beyond the site of DCMU action. We observed a slight decrease in DLE in the presence of 100μM of $PbCl_2$, and this decrease was enhanced in the presence of DCMU (a poison of photosynthesis). These

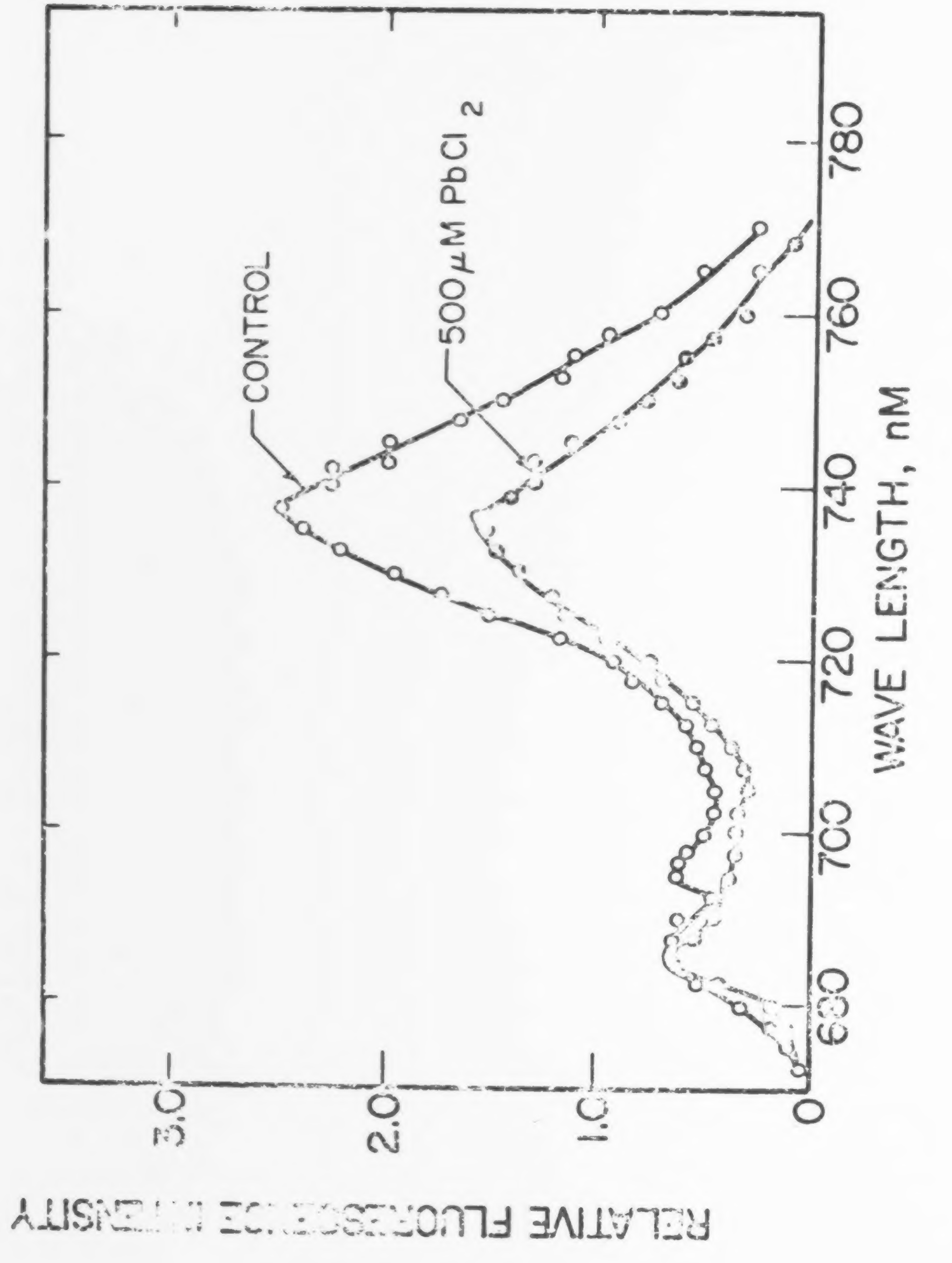

Figure 68. Emission spectra of Chl fluorescence at 77°K of normal and treated (500 μM $PbCl_2$) chloroplast suspensions. λ_{exc} = 436 nm.

results are complex and difficult to interpret at this stage. More experiments of this type will be performed in order to be able to reach any definite conclusion.

In conclusion, our experiments suggest that lead chloride affects photosynthesis in several ways. From measurements of photoreduction of DCPIP in isolated chloroplasts and the time course of Chl a fluorescence, lead chloride (50 μM - 500 μM) seems to inhibit photosystem II activity. However, at higher concentrations it probably feeds electrons to PS II. The last point needs to be confirmed by more experiments and using other approaches.

Measurements of emission spectra at 77°K of treated chloroplasts indicate that lead causes conformational changes of the membranes of the chloroplasts in such a way that spill-over of excess light energy from PS II to PS I is decreased. Furthermore, lead chloride seems to affect the trap of PS II.

No definite conclusion could be made at this point as to the effect of lead on PS I but our data indicate that even this reaction may be suppressed by the binding of lead with its enzymes. Experiments are in progress to give an answer to this question.

As for future experiments, we still have to test the effect of lead on photosynthesis using in vivo approach to introduce lead to chloroplasts, i.e., feeding lead salts through the roots of plants. This is an important point to investigate, i.e., to see if lead salts are also inhibiting photosynthesis in plants in the same fashion as when they are introduced directly to the reaction mixture of chloroplasts. We also plan to test the effect of other heavy metals like cadmium, copper and mercury on the light reactions of photosynthesis using the same approach we used to study the effect of lead on photosynthesis. We also plan to study the mechanism of action of these heavy metals in isolated chloroplasts of maize and in soybeans. Finally, in order to study the movement of heavy metals across cell walls and cell membranes, we plan to use single cells of algae and intact chloroplasts in the future.

C. Respiratory Effects of Trace Elements

Lead has been reported to adversely influence several mitochondrial enzymatic systems concerned with oxidation of succinic acid and NADH (Koeppe and Miller, 1970; Miller and Koeppe, 1971). Such effects were not observed in a reaction medium containing phosphate before Pb addition, and the Pb-induced inhibition of succinate oxidation was reversed by the subsequent addition of phosphate. While making these observations we also observed an accelerated swelling of isolated mitochondria in KCl reaction media due to Pb. Questions arose concerning the relationship of this swelling and the enzymatic inhibitions previously observed. In addition, questions presented themselves about the relationship of our Pb observations at the mitochondria level to a whole plant response, and also if we could learn more about Pb toxicity by studying the effects of other trace elements on the reaction of isolated corn mitochondria. Both tacks are being pursued as reported in the remainder of this section.

1. Effects on Excised Root Respiration

There was no consistent effect of relatively high Pb concentrations on the respiration of excised roots incubated with $PbCl_2$ for two hours (Table 35). Because of previously reported findings in uptake experiments, and the profound Pb-phospate interaction in isolated mitochondrial experiments, the root respiration data present no surprises. A likely hypothesis

Table 35. Effect of $PbCl_2$ on the O_2 uptake by excised corn roots. Primary roots of 3-day-old etiolated corn seedlings were excised and placed in aerated incubation media consisting of distilled water, 10^{-2} M $PbCl_2$, or 10^{-3} M $PbCl_2$. After 2 hr, 4 ml of the incubation media and 2 roots were placed in a 4 ml glass reaction vessel and a Clark O_2 electrode was placed in the top. Oxygen uptake from this solution was monitored polarographically for 15 min and recorded on a strip chart recorder. Values given represent averages of 4 individual determinations.

Treatment	Rate of O_2 uptake/min x g fresh weight	
	Experiment 1	Experiment 2
Check (distilled H_2O)	688	389
10^{-3} M $PbCl_2$	713	421
10^{-3} M $PbCl_2$	784	358

is that any Pb taken up during the incubation period was rendered inactive, probably as a lead-phosphate precipitate.

A number of experiments have been completed in which the Cd effects on excised roots respiration were monitored in a fashion similar to that previously reported for Pb. There has been some variability in our results, but with a two-hour preincubation time 10 mM Cd does seem to reduce the rate of O_2 uptake from solution from approximately 500 nmoles O_2/min x g fresh weight, to approximately 300 nmoles O_2/min x g fresh weight.

2. Lead Effects on Isolated Mitochondria

a. Soybean mitochondria. Since our initial experiments (Koeppe and Miller, 1970; Miller and Koeppe, 1971) had utilized isolated corn mitochondria, it was necessary to determine if our results were also applicable to isolated plant mitochondria from other species, or if they were unique for isolated corn mitochondria. On a qualitative basis, we have found that mitochondria isolated from soybean hypocotyls are comparable to isolated corn mitochondria in their behavior in the presence of Pb. Lead stimulated the oxidation of exogenous NADH, inhibited the oxidation of succinate, but had little effect on the oxidation of malate + pyruvate. Because of this preliminary evidence from experiments with isolated soybean mitochondria, it seemed that the effects of Pb on isolated corn mitochondria were not specific for corn but are probably true for plant mitochondria in general. We therefore decided to concentrate our efforts on isolated corn mitochondria, since we know more about them than most other plant mitochondria. In addition, the questions posed from our preliminary experiments definitely predicated more in-depth studies rather than a survey of Pb effects on a variety of isolated plant mitochondria. The remainder of mitochondrial studies reported here were completed utilizing corn mitochondria isolated according to the procedure of Miller, *et. al*. (1970).

All experiments were executed in 4 ml reaction media containing 200 mM KCl, 20 mM Tris-HCl (pH 7.5), and 1 mg/ml bovine serum albumin. Mitochondrial additions and other amendments were made to the reaction media as indicated in the tables and figures. Oxygen uptake was measured polarographically with a Clark oxygen electrode fitted into the top of the cell. The percent transmittance of light (520 mμ) through the mitochondrial reaction media solutions was measured on a Bausch and Lomb Spectronic 70 spectrophotometer. Both current outputs from the O_2 electrode and the spectrophotometer were simultaneously recorded on a dual channel recorder.

b. Lead-induced mitochondrial swelling. When plant mitochondria are placed from the 0.4 sucrose solution into which they are isolated into a 200 mM KCl solution, they swell due to the passive influx of K^+ and the concomitant movement of water. This influx can be reversed and an efflux initiated (contraction) when an oxidizable substrate is added. These fluxes can be monitored spectrophotometrically as changes in percent transmittance. It is often possible to correlate abnormal mitochondrial swelling with disruptions in the membrane and/or the respiratory and phosphorylative function of the membrane. Any changes in such percent transmittance due to the presence of Pb would likely represent Pb-membrane interactions.

Figure 69 illustrates the effect of varying concentrations of Pb on mitochondria actively oxidizing succinate or NADH. In both instances the higher concentrations of Pb caused pronounced swelling. As in our earlier studies (Koeppe and Miller, 1971; Koeppe, *et. al*., 1972), the rapid swelling correlates with an inhibition of the rate of succinate oxidation and an increase in the rate of NADH oxidation. Lead had little effect on the rate

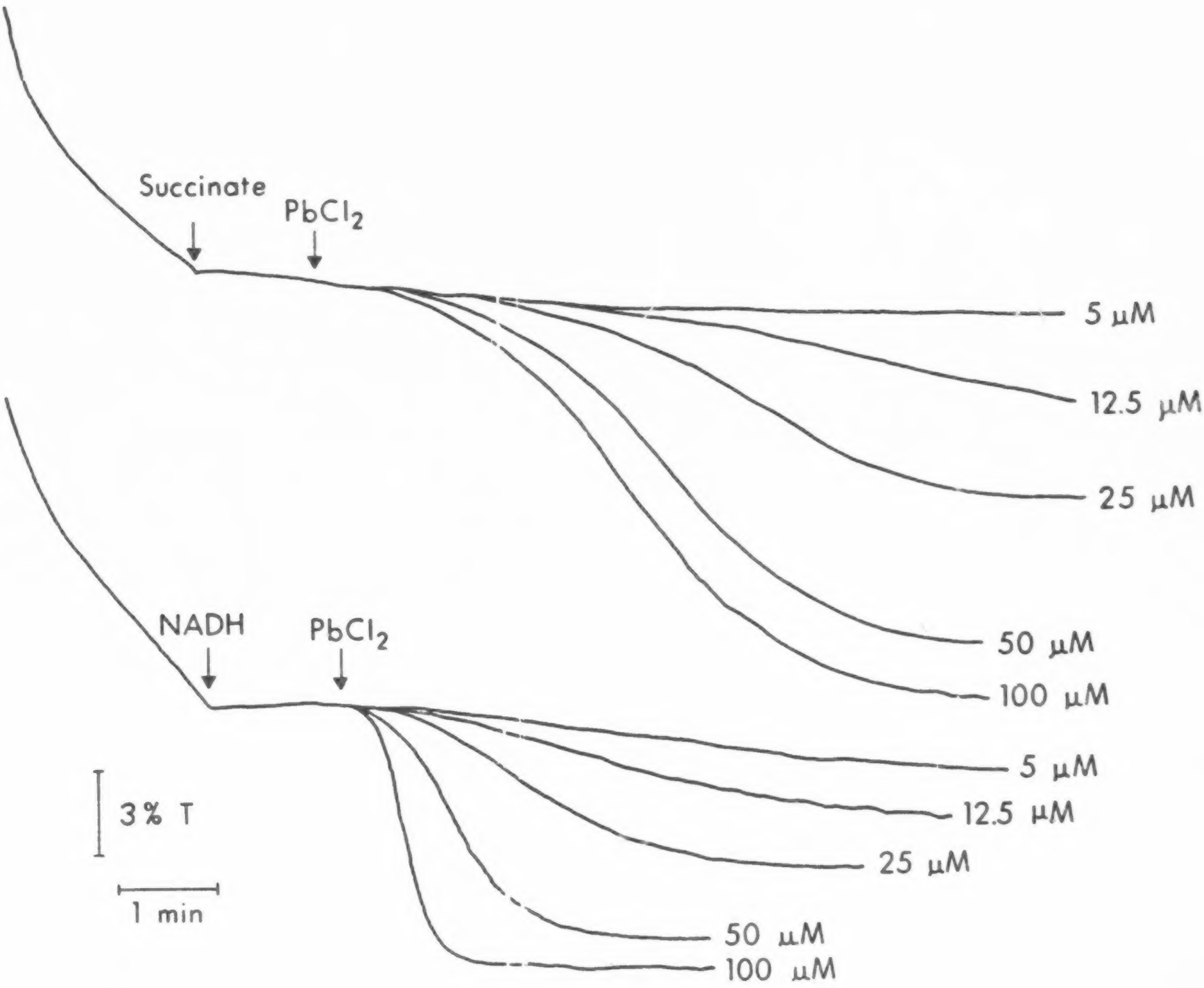

Figure 69. The effect of varying $PbCl_2$ concentrations on the swelling of isolated mitochondria under active conditions. Reaction media conditions were as given in section C-2 . Additions of $PbCl_2$ were made at the point indicated to give the final concentrations indicated at the end of the swelling trace. Substrate additions were 40 µmoles succinate or 2 µmoles NADH. A downward deflection in the percent transmittance traces represents increased transmittance of light through the mitochondrial suspension, or swelling.

of oxidation of malate (Miller and Koeppe, 1971), and Pb additions in the presence of malate + pyruvate did not induce swelling (Figure 70). Figure 70 also indicates that Pb enhanced the rate of swelling in the absence of any oxidizable substrate. The initial addition of 4 mM KH_2PO_4 (Pi) inhibited the effect of Pb on swelling as it had on substrate oxidation (Koeppe and Miller, 1970; Miller and Koeppe, 1971). However, there was no reversal of Pb-induced swelling if the Pi was added after the Pb, even though the inhibition of succinate oxidation was relieved by the Pi. This last experiment points to the likelihood that effects of Pb on swelling, and on the rate of oxidation of succinate, are not directly related but are rather isolated simultaneous events.

c. Mitochondrial uptake and/or adsorption of Pb. The results presented in Table 36 are preliminary, even though the experiments have been repeated several times. In the experiments mitochondria were treated and then suspended on a 0.8 μ Millipore filter. After washing, the material suspended on the filter was ashed and Pb analyses made. Results are presented as μg Pb/mg mitochondrial protein. When no mitochondria were present, additions were made into comparable solution volumes and the results are presented as if the mitochondria were present, thus making all the data comparable.

In the absence of substrate mitochondria bound more Pb when Pi was present than when it wasn't. Succinate in the absence of Pi had little effect on Pb binding, but in the presence of Pi the Pb bound was more than 3 times greater than it was with Pi, but without succinate. This figure is, however, somewhat negated by the observation that nearly as much Pb was found on the filter when mitochondria were left out of the succinate-Pi-Pb solution as when they were present. A preliminary guess might be that there is some

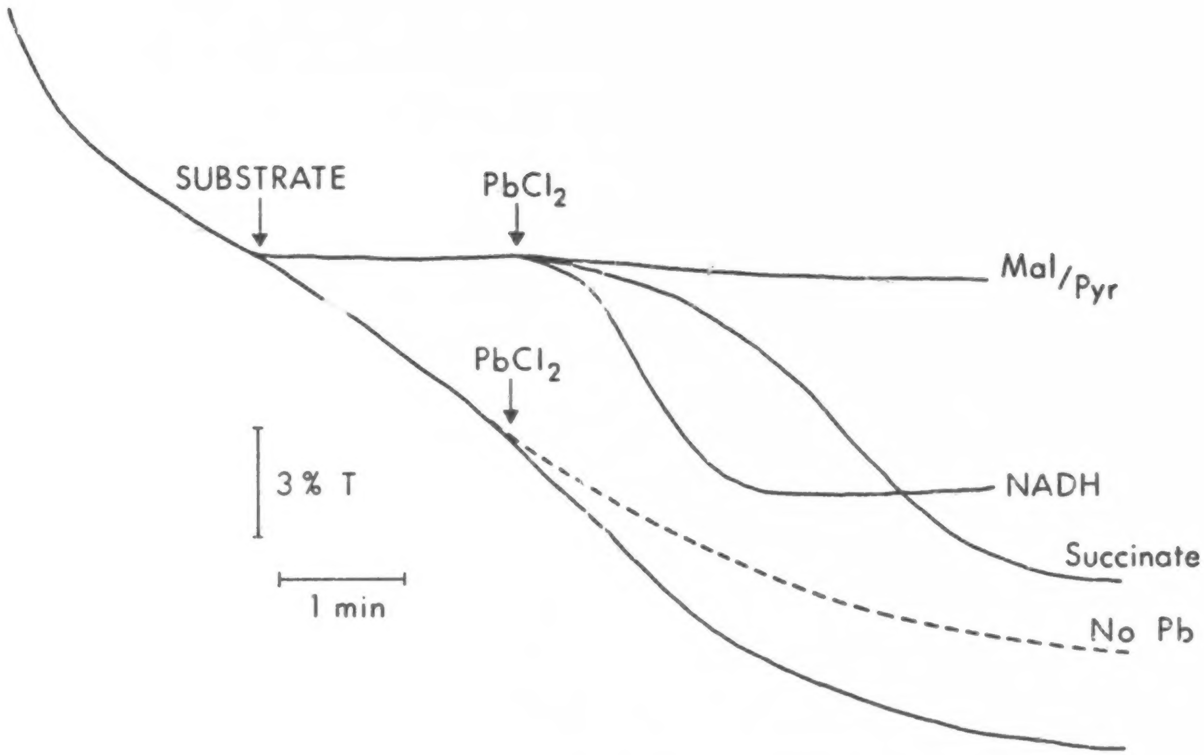

Figure 70. The effect of $PbCl_2$ on mitochondrial swelling under passive and active conditions. Reaction media conditions were as indicated in section C-2. Substrate additions were 40 μmoles malate + 40 μmoles pyruvate, 40 μmoles succinate, or 2 μmoles NADH at the point indicated. The lower 2 traces represent swelling in the absence of substrate. A $PbCl_2$ addition to give a reaction media concentration of 50 μM was made as indicated. A downward deflection of the percent transmittance traces represents increased light transmittance, or swelling.

Table 36. The uptake and/or adsorption of Pb by isolated mitochondria. Reaction media consisted of 200 mM KCl, 20 M Tris-HCl (pH 7.5), 1 mg/ml (4 ml cell volume) bovine serum albumin and 4 mM KH_2PO_4 as indicated. Mitochondria were added to the reaction vessel and subsequent additions of substrate and $PbCl_2$ (50 μM) were made at 2 and 4 min respectively. Oxygen uptake from the reaction media was measured by a Clark O_2 electrode. Eight min after the initial addition of mitochondria the cell contents were poured onto a 0.8 μ Millipore filter and washed with at least 10 ml of 0.4% sucrose. The Millipore filter + mitochondria was wet ashed, and Pb concentrations were determined.

Reaction media	Substrate	Effect of Pb and O_2 uptake % of control	μg Pb/mg Protein
-P	---	---	0.91
+P	---	---	1.10
-P	Succinate	---	0.91
+P	Succinate	---	3.65
Mito -P	Succinate	25.9	6.04
Mito +P	Succinate	100.0	39.59
Mito +P	---	---	14.62
Mito -P	---	---	7.82

sort of a Pb-Pi-succinate complex found under our reaction media conditions. Because of the effects and interactions of Pb, Pi, and succinate on mitochondria function as mentioned previously, such a complex could be of considerable physiological importance.

D. Effects of Cadmium on Isolated Mitochondria

Since Pb has been found to be such a potent effector of mitochondrial reactions, a natural question was to wonder if other trace elements present as pollutants might not also be equally as effective. The work with Cd presented here, and Ni later, leaves little question that at least these two trace elements are potentially dangerous if moved within plants to mitochondrial sites.

1. Electron transport

Electron flow from 3 oxidizable substrates to O_2 was measured by measuring O_2 uptake from the reaction media. In the absence of Pi (Figure 71), Cd stimulated the oxidation of exogenous NADH at low concentrations, but inhibited it at higher concentrations. The oxidation of both malate + pyruvate and succinate was inhibited by Cd (Figure 71), with succinate oxidation being most adversely affected. In the presence of Pi (Figure 72) the Cd-induced inhibition of substrate oxidation was reduced at the lower Cd concentrations. Interestingly, the oxidation of succinate was stimulated by 0.1 mM $CdCl_2$ in the presence of Pi, an observation for which we have no ready explanation.

Through the use of dyes which accept electrons early in electron transport, probably before the cytochrome b complex, it is possible to differentiate the general site of Cd activity. Figure 73 presents comparative

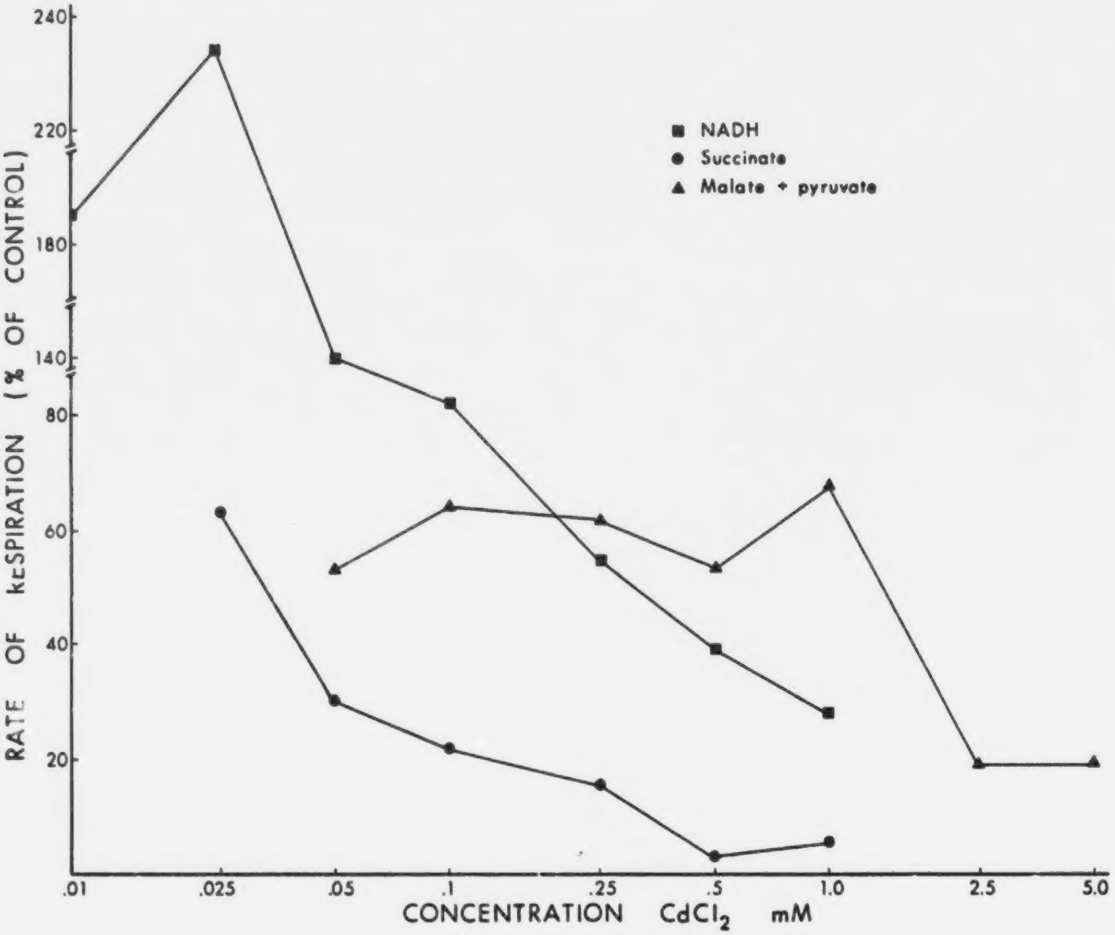

Figure 71. The effect of varying concentrations of $CdCl_2$ on the rate of mitochondrial respiration in the absence of phosphate. Substrate (2 µmoles NADH, 40 µmoles succinate, or 40 µmoles malate + 40 µmoles pyruvate) was added to a mitochondrial-reaction media suspension 2 min. after the initial addition of mitochondria. After a discernable rate of O_2 uptake had been established (1-2 min.) $CdCl_2$ was added and the new rate of O_2 uptake determined. The data given are a comparison of the second rate after $CdCl_2$ addition to the rate before Cd addition. Reaction media conditions were as given in section C-2 .

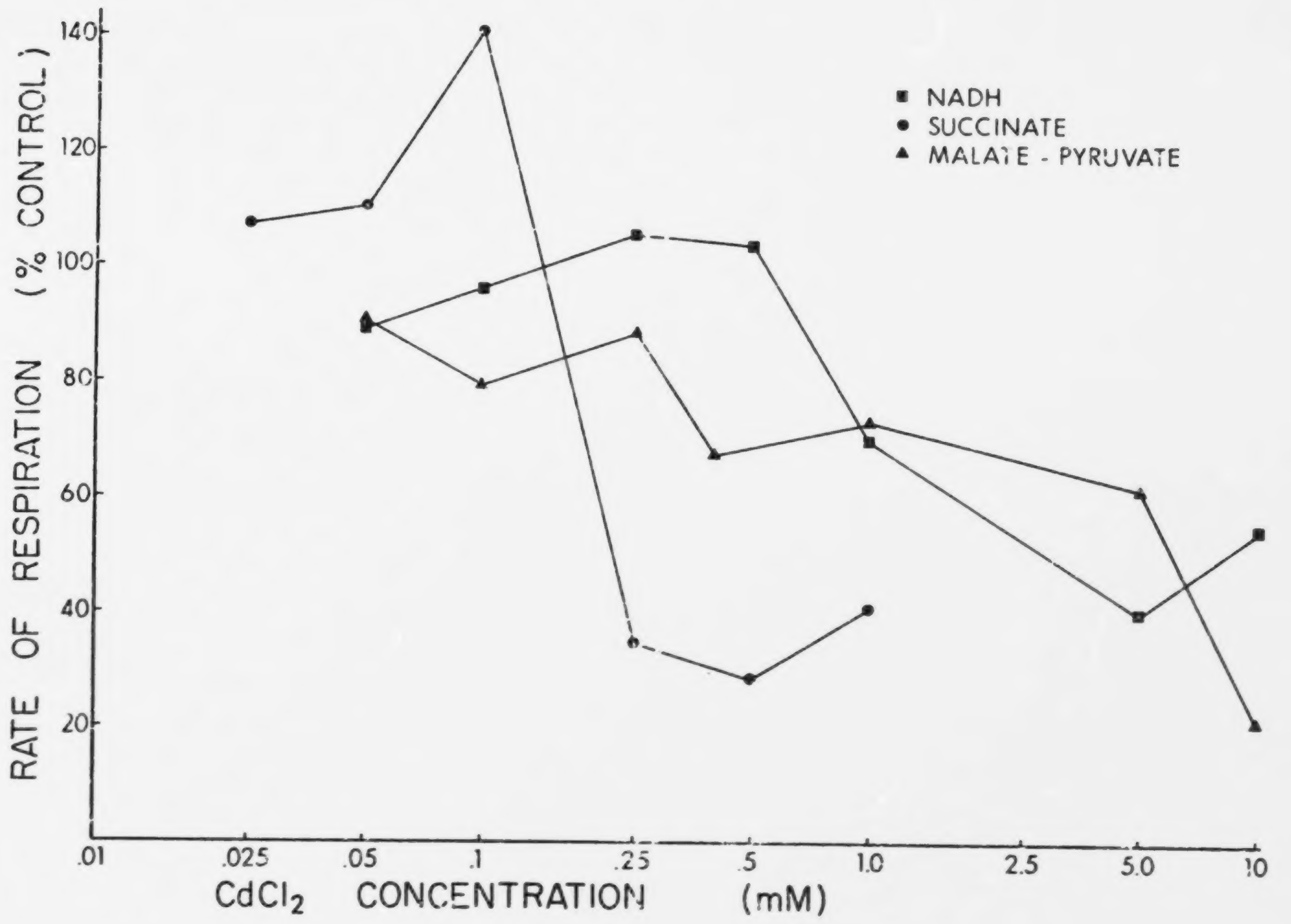

Figure 72. The effect of varying concentrations of $CdCl_2$ on the rate of mitochondrial respiration in the presence of phosphate. Mitochondria were taken through a substrate state 4--state 3--state 4 cycle through the addition of 300 μmoles ADP after substrate (2 μmoles NADH, 40 μmoles succinate, or 40 μmoles malate + 40 μmoles succinate) addition. $CdCl_2$ was added to give the concentrations indicated after the state 4 rate of O_2 uptake had been determined. The new rate of O_2 uptake in the presence of $CdCl_2$ was established with the data given being a comparison of the rate after $CdCl_2$ addition to the rate before addition. Reaction media conditions were as indicated in section C-2, but with the addition of 4 mM Pi.

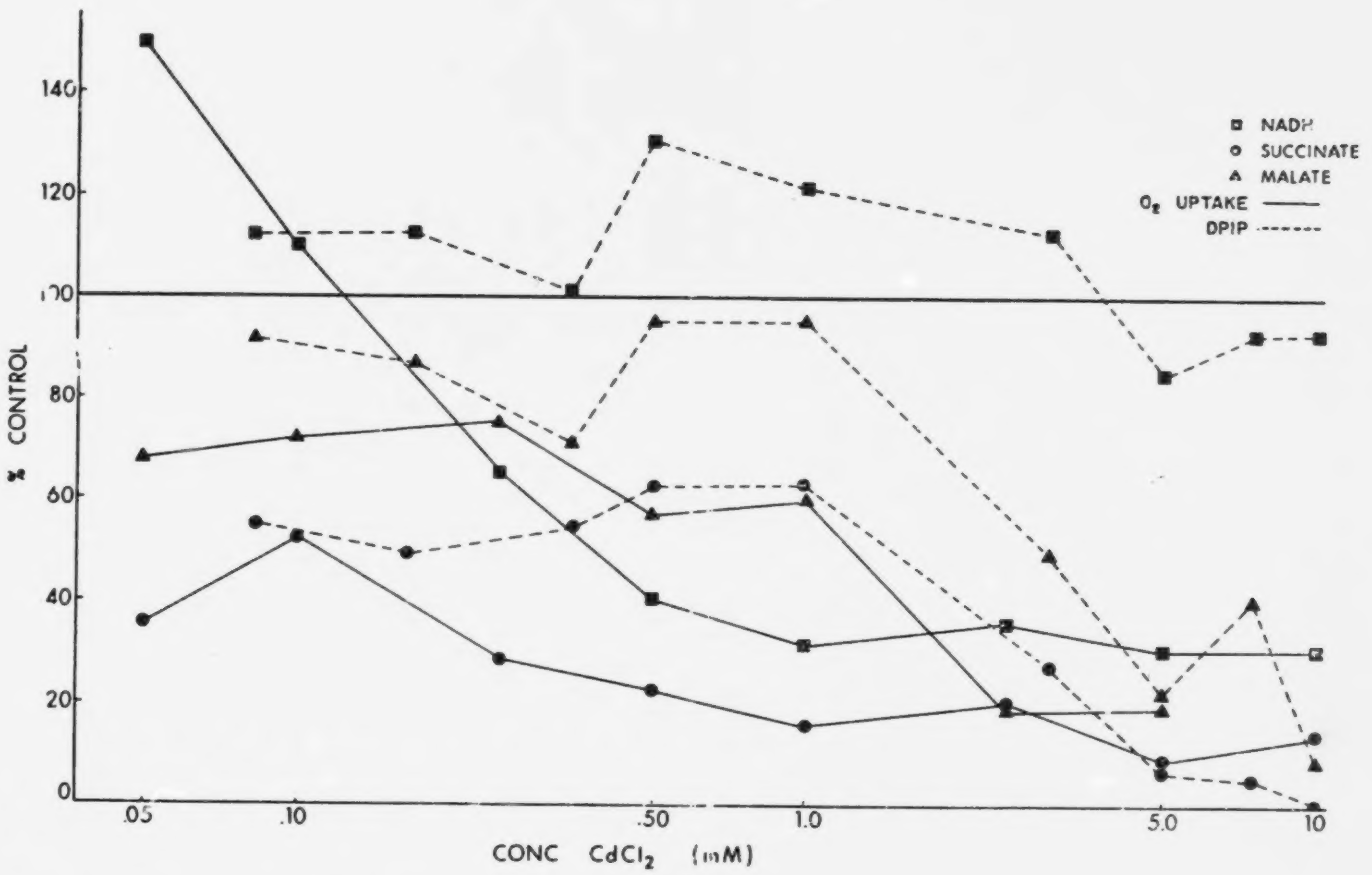

Figure 73. A comparison of the effects of $CdCl_2$ on the rate of electron flow from substrate to oxygen and from substrate to 2,6-dichlorophenolindophenol (DCPIP). Reaction media conditions were as given in section C-2, and O_2 uptake data were obtained as given in Figure 71. It should be noted that malate (40 μmoles) was used in this study, not malate + pyruvate. Rates of DCPIP reduction were measured spectrophotometrically as previously described (Koeppe and Miller, 1972).

data on the effects of Cd on O_2 uptake (involving the entire electron transport chain) and the reduction of the dye 2,6-dichlorophenol-indophenol (DCPIP). It should be noted that the oxidation of malate, not malate + pyruvate, was monitored in these experiments. The close correlation of the Cd-induced inhibition of malate and succinate oxidation (O_2 uptake) and the inhibition of the reduction of DCPIP leads to the tentative hypothesis that the site of Cd inhibition is probably before the cytochrome b complex, and most likely with the initial dehydrogenase enzymes. Such a correlation with NADH, however, was not observed and may be significant. More probably, however, the nonspecificity of this diaphorase reaction (4-5 different enzymes could be involved) is a factor and the effects of Cd on the one dehydrogenase enzyme involved in NADH-O_2 electron flow are clouded by this nonspecificity.

The Pb inhibition of succinate oxidation was relieved by subsequent addition of Pi. Figure 74 presents O_2 uptake traces indicating the effects of Cd and Pi additions on succinate oxidation. These traces again demonstrate that Pi addition before Cd does decrease the effect of Cd. However, unlike the Pb instance, Pi addition after Cd did not relieve the Cd inhibition. Thus, it seems reasonable to infer that the Pi-Cd interaction is external to the mitochondria and likely not of the type observed with Pb. This argument is strengthened by the observations reported in Table 37. These experiments are comparable to those discussed previously for Pb and reveal virtually no Cd-Pi-succinate-mitochondrial binding interaction, quite unlike the results for Pb. When NADH was the oxidizable substrate, however, Pi did increase the rate of oxidation after a slight Cd inhibition (Figure 75). The significance, and relevance, of these data is not known at this time.

2. Swelling-contraction

The swelling induced by varying concentrations of Cd added to

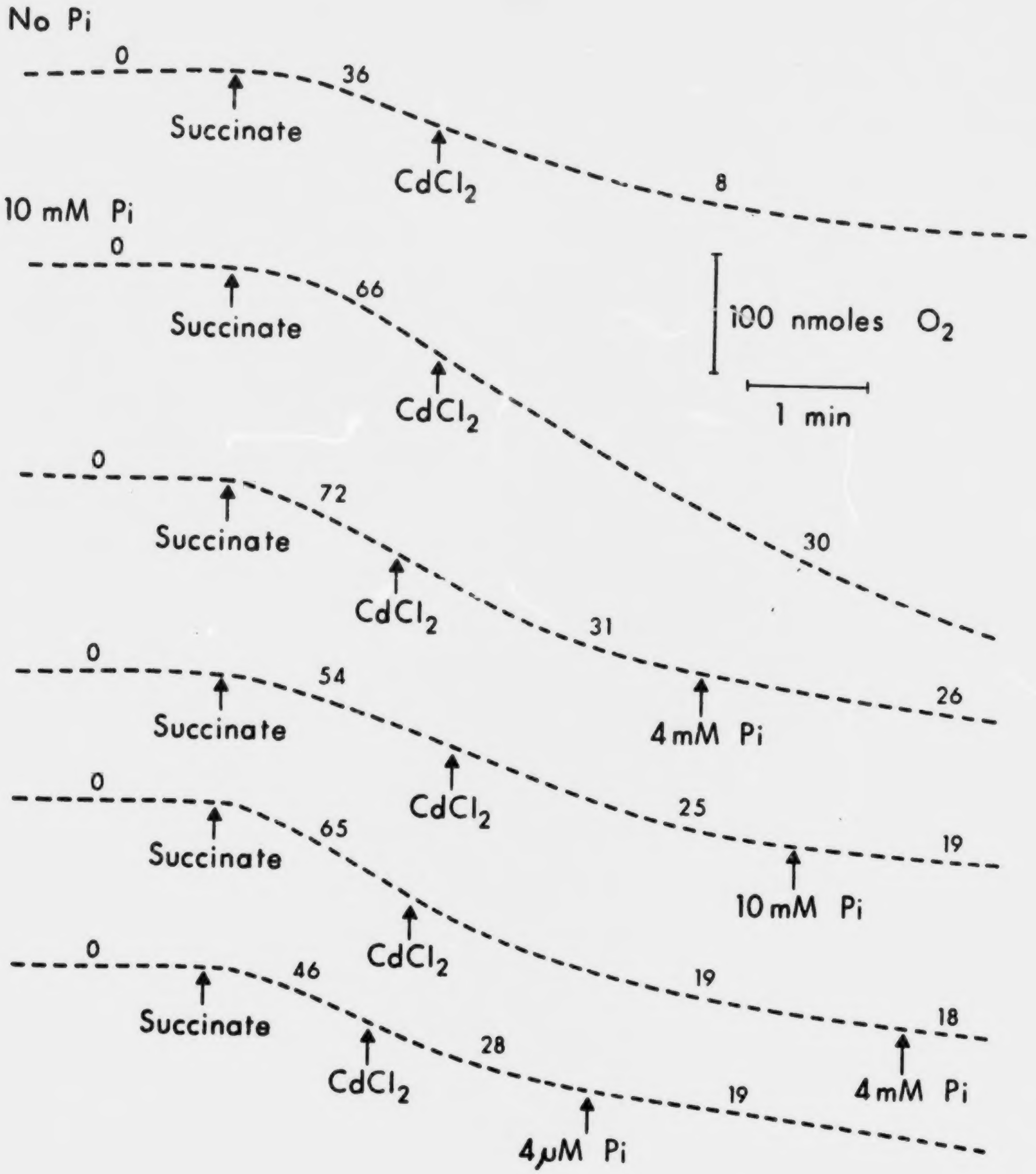

Figure 74. The effect of varying time and concentration additions of phosphate (Pi) on the rate of $CdCl_2$ influenced succinate oxidation. Additions of 40 μmoles succinate, $CdCl_2$ (0.1 mM reaction vessel concentration), or Pi were made as indicated. Reaction media conditions were as given in section C-2 . Numbers next to traces represent rates of O_2 uptake expressed as nmoles O_2/min x mg mitochondrial protein.

Table 37. The uptake and/or adsorption of Cd by isolated mitochondria. Reaction media consisted of 200 mM KCl, 20 mM Tris-HCl (pH 7.5), 1 mg/ml (4 ml cell volume) bovine serum albumin and 4 mM KH_2PO_4 as indicated. Mitochondria were added to the reaction vessel and subsequent additions of substrate and $CdCl_2$ (0.1 mM) were at 2 and 4 min respectively. Oxygen uptake from the reaction media was measured by a Clark O_2 electrode. Eight min after the initial addition of mitochondria the cell contents were poured onto a 0.8 μ Millipore filter and washed with at least 10 ml of 0.4% sucrose. The Millipore filter + mitochondria was wet ashed, and Cd concentrations were determined.

Reaction media	Substrate	Effect of Cd on O_2 uptake % of control	μg Cd/mg Protein
-P	---	---	0.57
+P	---	---	0.57
Mito -P	---	---	2.02
Mito +P	---	---	1.56
Mito -P	Succinate	25.0	1.67
Mito +P	Succinate	45.5	1.53
Mito -P	NADH	73.3	1.92
Mito +P	NADH	90.9	1.63
Mito -P	ml/pyr	71.4	1.17
Mito +P	ml/pyr	100.00	0.89

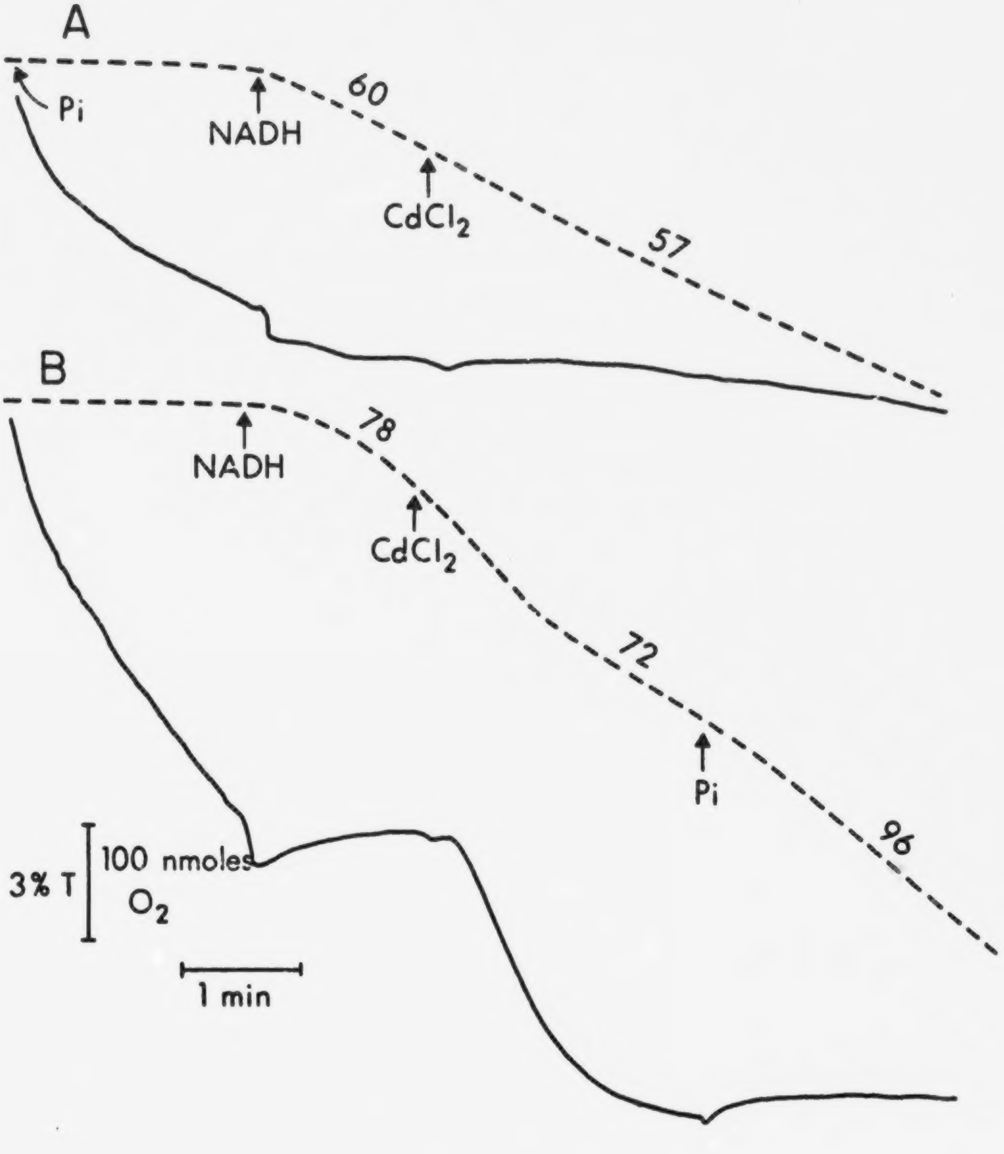

Figure 75. The effect of Phosphate (Pi) on $CdCl_2$ induced changes in the rate of NADH oxidation and concomitant swelling. The dashed line represents the O_2 electrode trace (a downward deflection represents a decrease in O_2 concentration in the reaction medium, or O_2 uptake by mitochondria) and the solid line represents % transmittance changes (a downward deflection represents swelling). Additions of Pi (to give 4 mM concentration), NADH (2 μmoles), to $CdCl_2$ (to give 0.1 mM concentration) were made as indicated. Rates of O_2 uptake are given as nmoles O_2/min x mg mitochondria protein.

contracted mitochondria is reported in Figure 76. Mitochondria oxidizing either succinate or exogenous NADH exhibited appreciable swelling with low concentrations of Cd, and more swelling as Cd concentrations were increased. In the presence of malate + pyruvate, however, 1 mM Cd had no effect on swelling, which was quite unlike the succinate and NADH cases. It is interesting to note that this Cd concentration (1 mM) did occasion about a 30% reduction in the oxidation rate of malate + pyruvate (Figure 76).

Figures 77 and 78 present data showing the involvement of Pi with swelling under active conditions. Phosphate was observed to reduce the Cd-effected swelling when succinate or exogenous NADH were being oxidized. The degree of reduction increased as Pi concentrations were increased. At the same time, the concomitant rates of O_2 uptake show a reduction in the Cd-induced inhibition of O_2 uptake which parallels the Pi-effected reduction in Cd-induced swelling.

The effects of Cd on passive mitochondrial swelling are presented in Figure 79. These traces generally portray an effect of Cd on swelling similar to that observed under active conditions. Cadmium additions accelerated swelling in the absence of Pi, but not in its presence.

3. Energy coupling

In intact cells the flow of electrons through the electron transport chain is coupled to a phosphorylation process producing ATP. Isolated mitochondria, too, have the capacity to carry on oxidative phosphorylation, even though the experiments reported up to this time were conducted under nonphosphorylating conditions. A measure of the efficiency of coupling can be obtained by calculating a respiratory control ratio (RCR). By definition this is the ratio of the rate of O_2 uptake when isolated mitochondria are

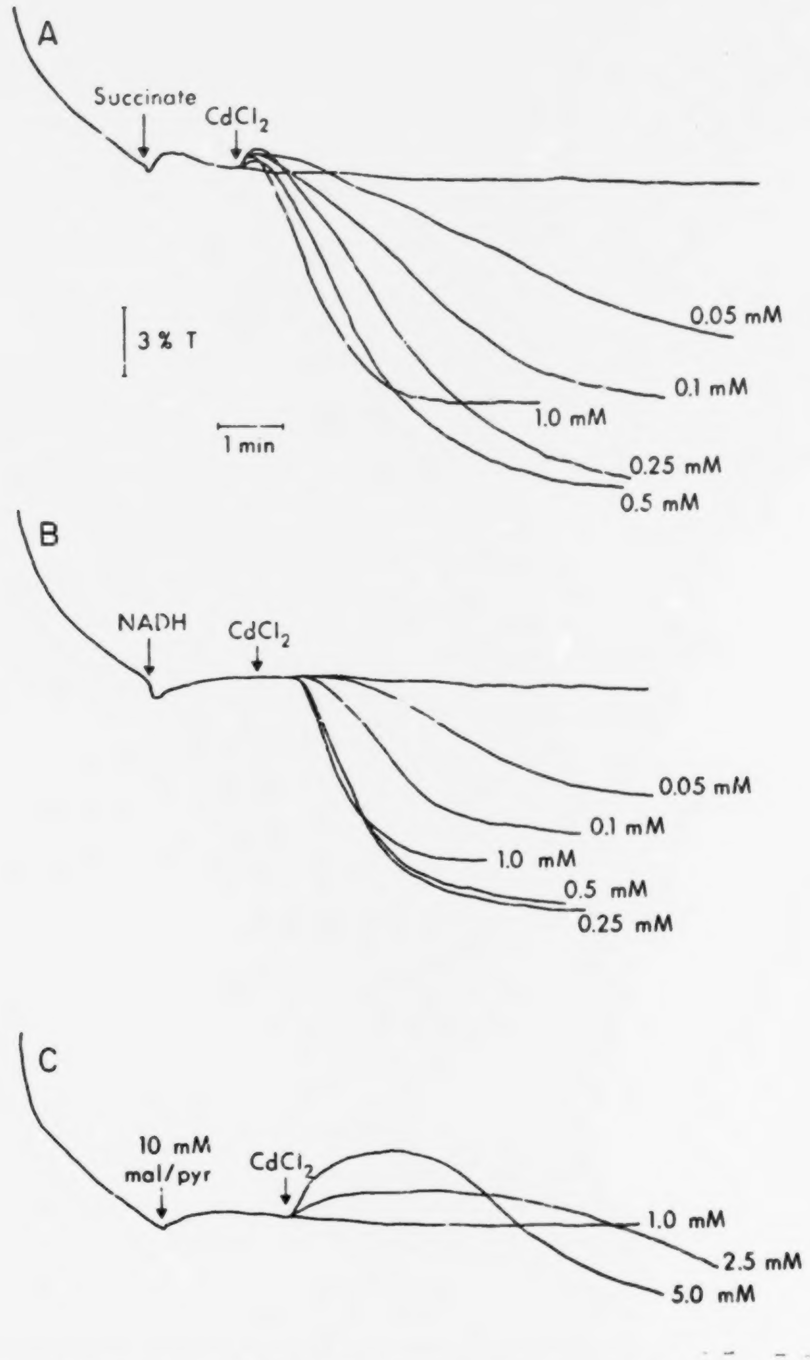

Figure 76. The effect of varying $CdCl_2$ concentrations on the swelling of isolated mitochondria under active conditions. Reaction media conditions were as given in section C-2. Additions of $CdCl_2$ were made at the point indicated to give the final concentration indicated at the end of the swelling trace. Substrate additions were 40 μmoles succinate, 40 μmoles malate + 40 μmoles pyruvate, or 2 μmoles NADH. A downward deflection in the percent transmittance trace represents swelling.

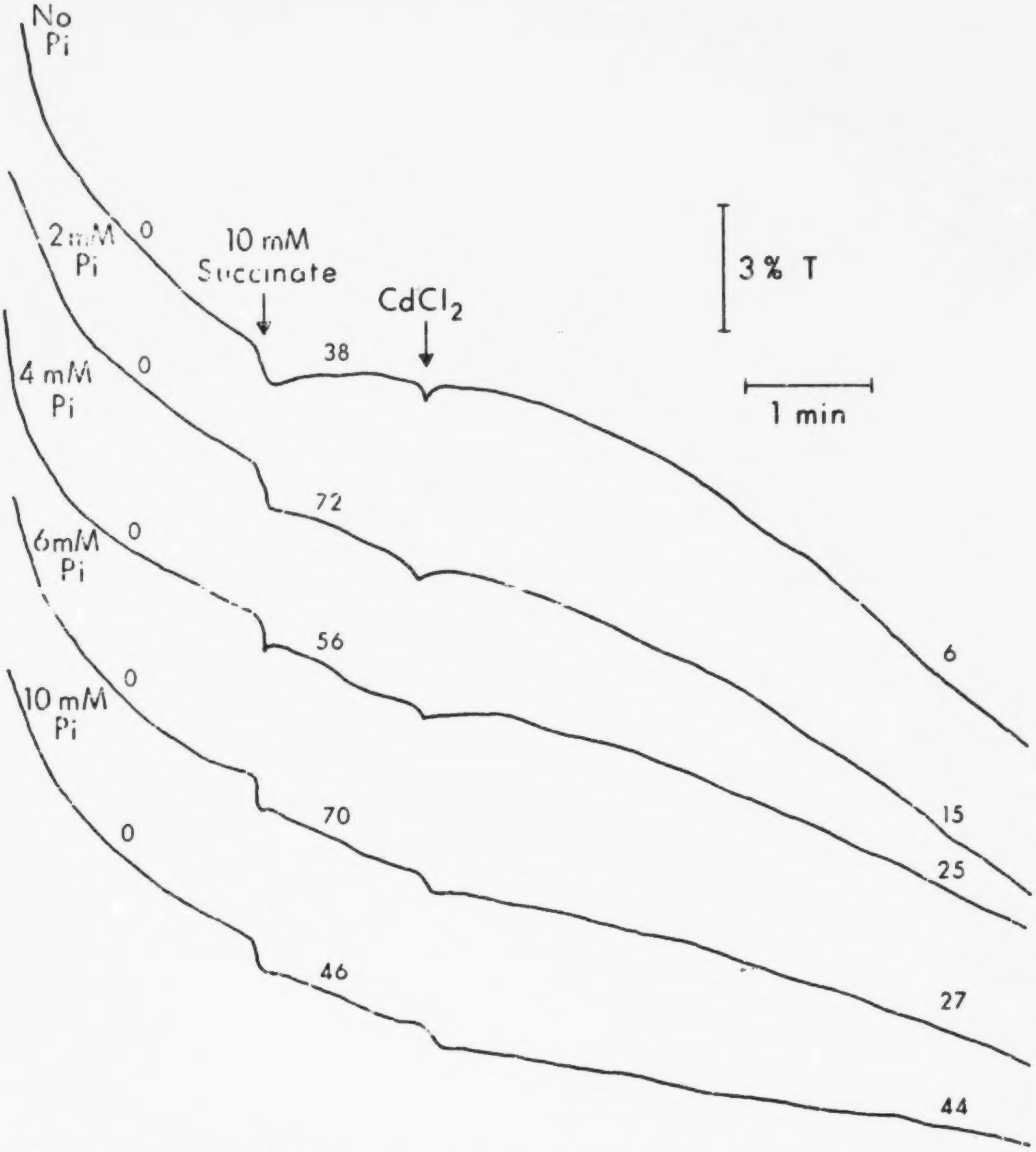

Figure 77. The effect of varying concentrations of phosphate (Pi) on the $CdCl_2$-induced swelling of mitochondria oxidizing succinate. Reaction media conditions were as given in section C-2. Additions to succinate (to give 10 mM concentration) and $CdCl_2$ (to give 10 mM concentration) were made as indicated. Traces represent percent transmittance changes with a downward deflection representing swelling. Numbers next to traces represent concomitant O_2 uptake rates and are given as nmoles O_2/min x mg mitochondrial protein.

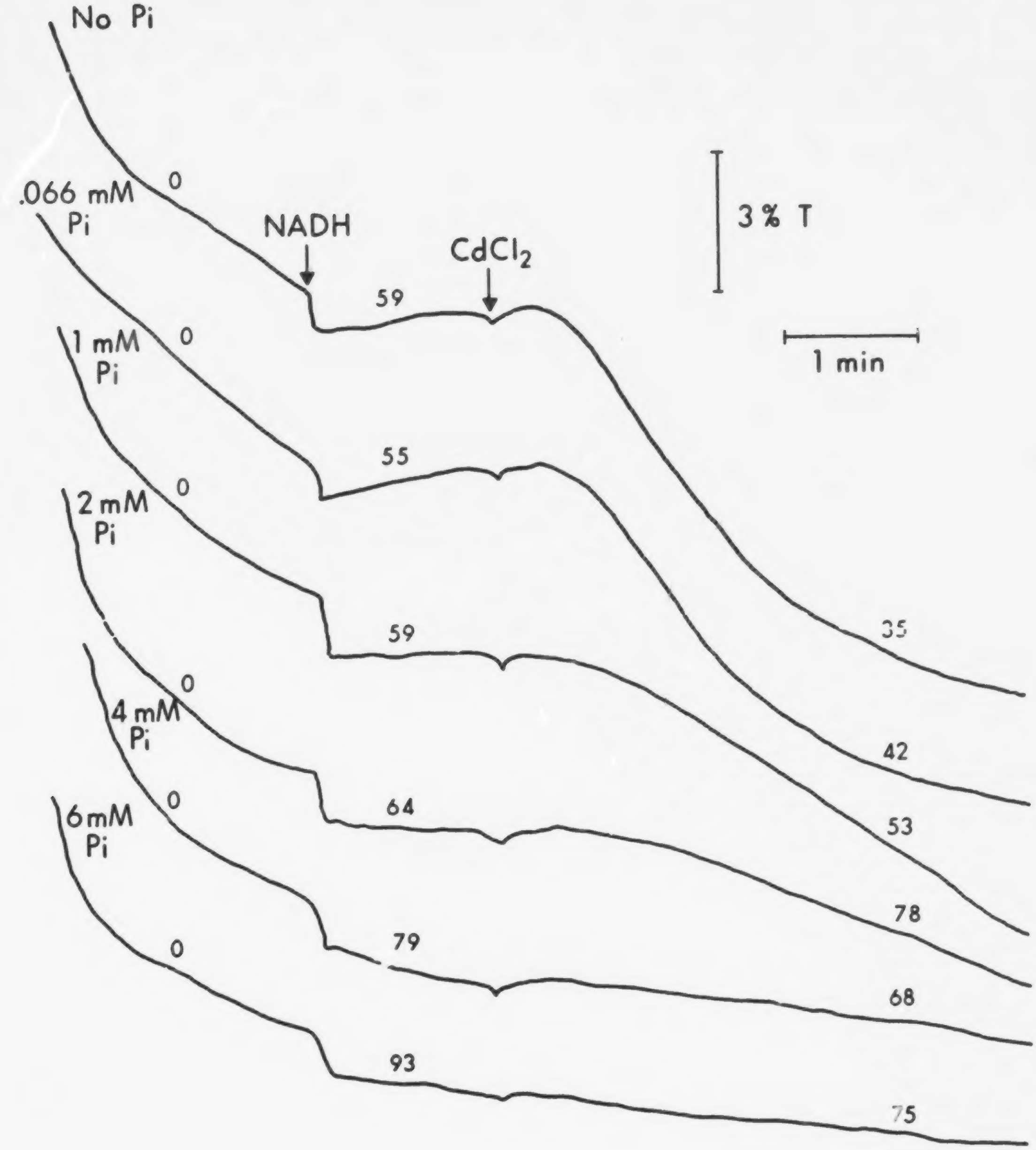

Figure 78. The effect of varying concentrations of phosphate (Pi) on the CdCl$_2$-induced swelling of mitochondria oxidizing exogenous NADH. See Figure 77 for details.

producing ATP to the rate when they are not producing ATP. The effect of an extraneous agent such as Cd on this ratio can be obtained by getting a RCR, adding Cd, then attempting to obtain a second RCR by adding ADP, which is converted to ATP. The data in Figure 80 indicate that Cd does reduce the RCR as Cd concentrations are increased. As in the previously reported studies, Cd was least effective on a system oxidizing malate + pyruvate.

A second measure of the efficiency of phosphorylation by isolated mitochondria is a ratio of ADP used to produce ATP to O atoms consumed in the concomitant electron transport process. Again, to measure the effects of Cd, a first ADP/O ratio can be determined, Cd added, and then a second ratio ascertained. Figure 81 presents these data which again exemplify a profound Cd effect at 0.25 - 0.5 mM when succinate or NADH were being oxidized. As expected, phosphorylation in association with the oxidation of malate + pyruvate was affected by Cd only at considerably higher concentrations.

The greater effect of Cd on phosphorylative systems functioning in conjunction with the oxidation of succinate or exogenous NADH when compared to systems oxidizing malate + pyruvate is not readily explainable. While the number of phosphorylative sites in the passage of electrons from these 3 oxidizable substrates is 2 for succinate or exogenous NADH, and 3 for malate, this should not have been a factor since we were calculating ratios and our data are presented as ratios. In addition, there is little evidence from the literature that there is any difference in the phosphorylative systems functioning in the presence of any oxidizable substrate. Thus, it does seem plausible to hypothesize some extramitochondrial nontoxic interaction between Cd and the malate + pyruvate which keeps Cd from reaching an effective site associated with the mitochondria. This, however, is mere conjecture and seems somewhat unlikely.

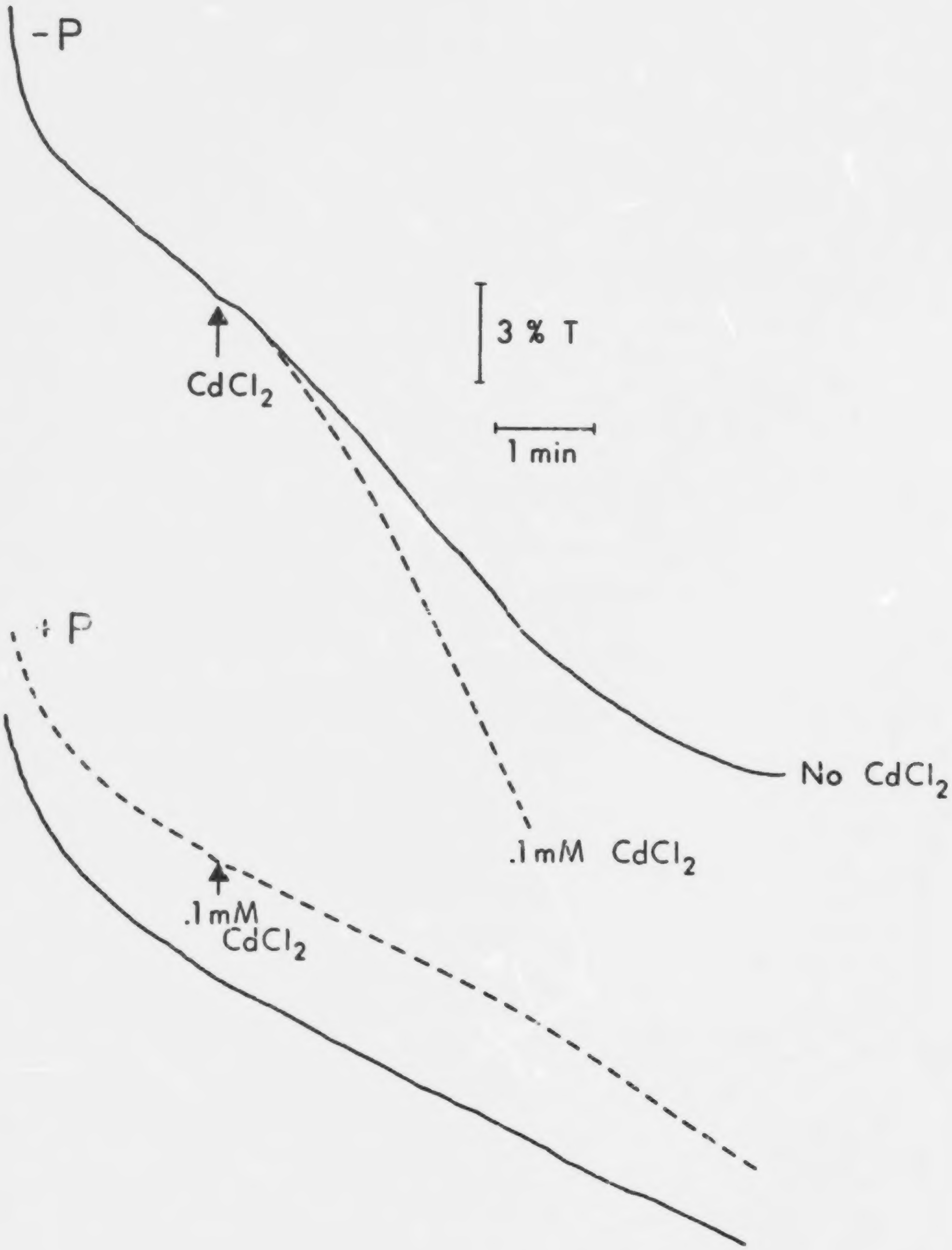

Figure 79. The effect of phosphate on the CdCl$_2$-induced increase in mitochondrial swelling under passive conditions. Traces represent percent transmittance with a downward deflection representing swelling. Reaction media conditions were as given in section C-2, but with the addition of 4 mM Pi as indicated. CdCl$_2$ additions gave a final concentration of 0.1 mM.

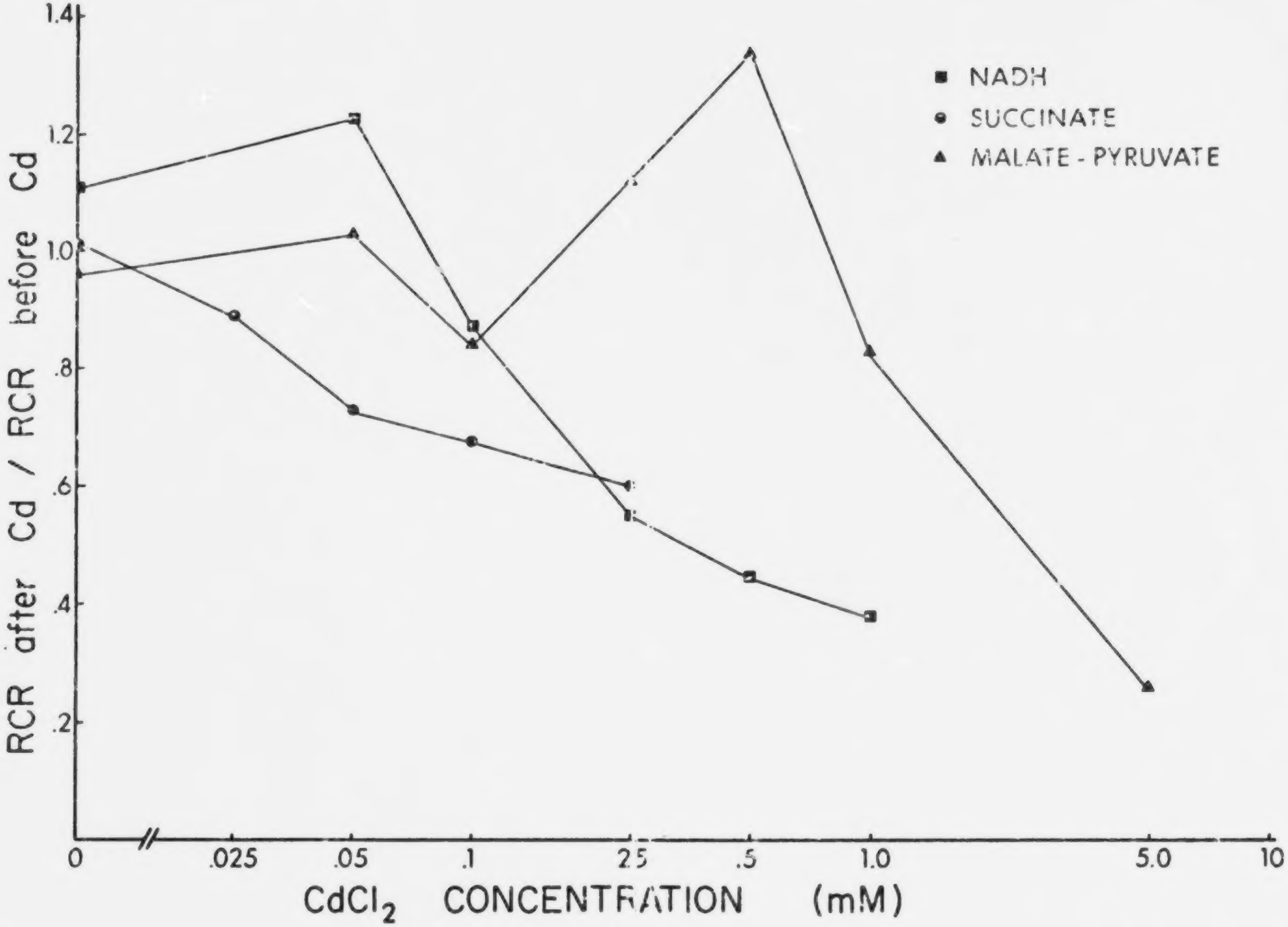

Figure 80. The effect of varying $CdCl_2$ concentrations on mitochondrial coupling, expressed as respiratory control ratios (RCR). Reaction media conditions were as given in Section C-2, but with the addition of 4 mM Pi. Substrate additions were 40 μmoles malate + 40 μmoles pyruvate, 40 μmoles succinate, or 2 μmoles NADH. See Section C-2 for experimental details.

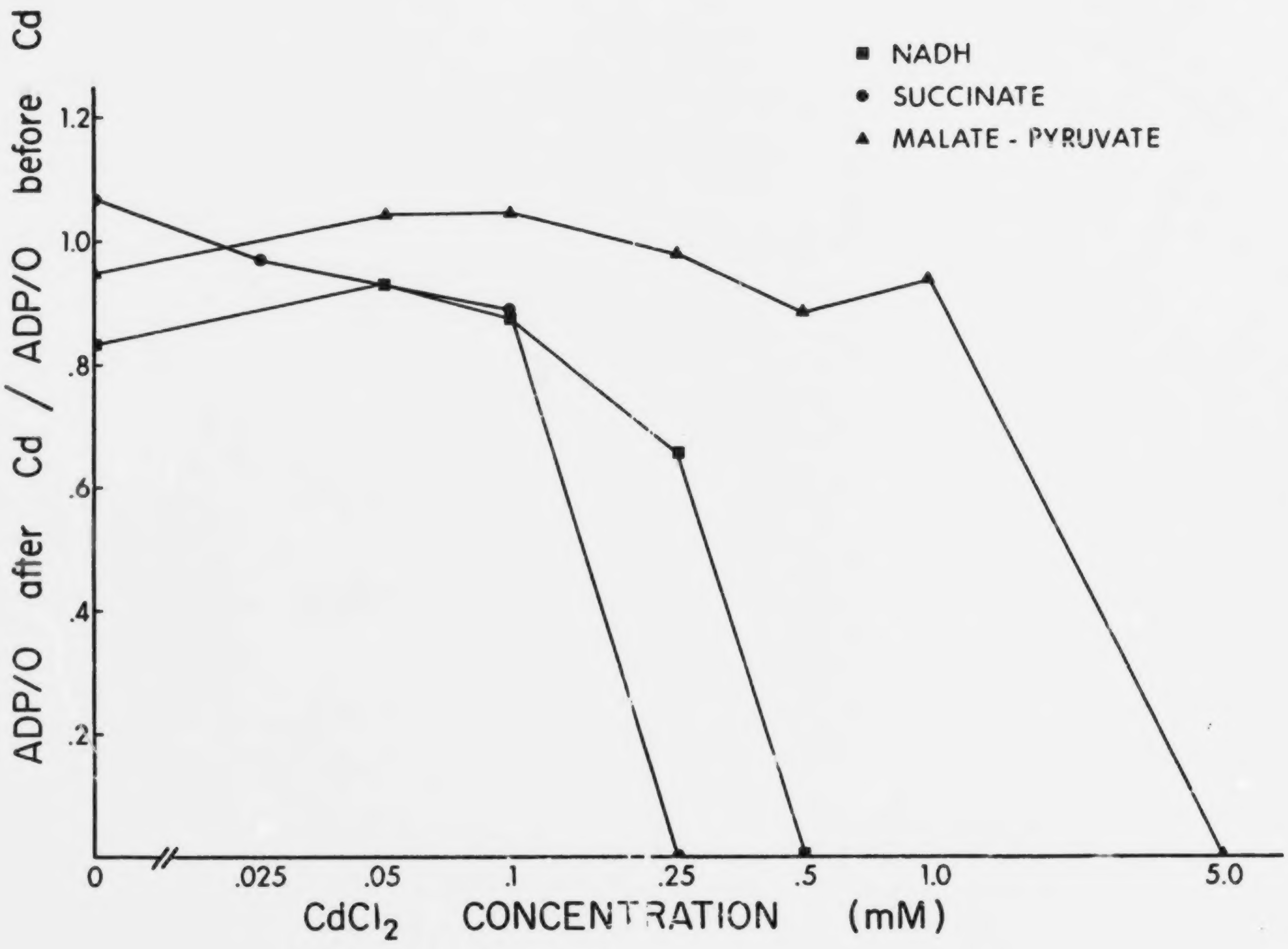

Figure 81. The effect of varying $CdCl_2$ concentrations on mitochondrial coupling expressed as ADP/O ratios. See Figure 80 for details.

E. Effects of Nickel on Isolated Mitochondria

1. Electron transport

As with both Cd and Pb, Ni produced a pronounced inhibition of the rate O_2 uptake from mitochondrial systems oxidizing succinate or exogenous NADH (Figure 82), and a lesser inhibition of malate + pyruvate oxidation. However, unlike the 2 previous trace elements studied, effective Ni concentrations were close to an order of magnitude greater, and, with the exception of malate + pyruvate oxidation, the presence of Pi had little effect.

When O_2 uptake (involving the entire electron transport chain) is compared with the reduction of DCPIP it again appears, as with Cd, that Ni is inhibiting electron transport early in the sequence of electron transfers (Figure 83). The 20% reduction in DCPIP reduction in the presence of NADH could be significant and likely indicates a Ni site of activity with NADH oxidation that is also before the cytochrome b complex.

2. Swelling

Nickel concentrations comparable to those of Pb or Cd which caused swelling (1 mM for succinate, 1 mM for NADH, and 5 mM for malate + pyruvate) did not effect any swelling under either active (Figure 84) or passive conditions.

At first glance the lack of Ni-induced swelling and the presence of Ni respiratory inhibitions suggest further that the swelling and respiratory inhibitions induced by Pb or Cd could be isolated but simultaneous events. However, caution must be exerted here since so little is known of the exact nature of the swelling event, and if all percent transmittance changes indicating swelling are indeed synonomous, electron microscopic pictures should help us resolve this.

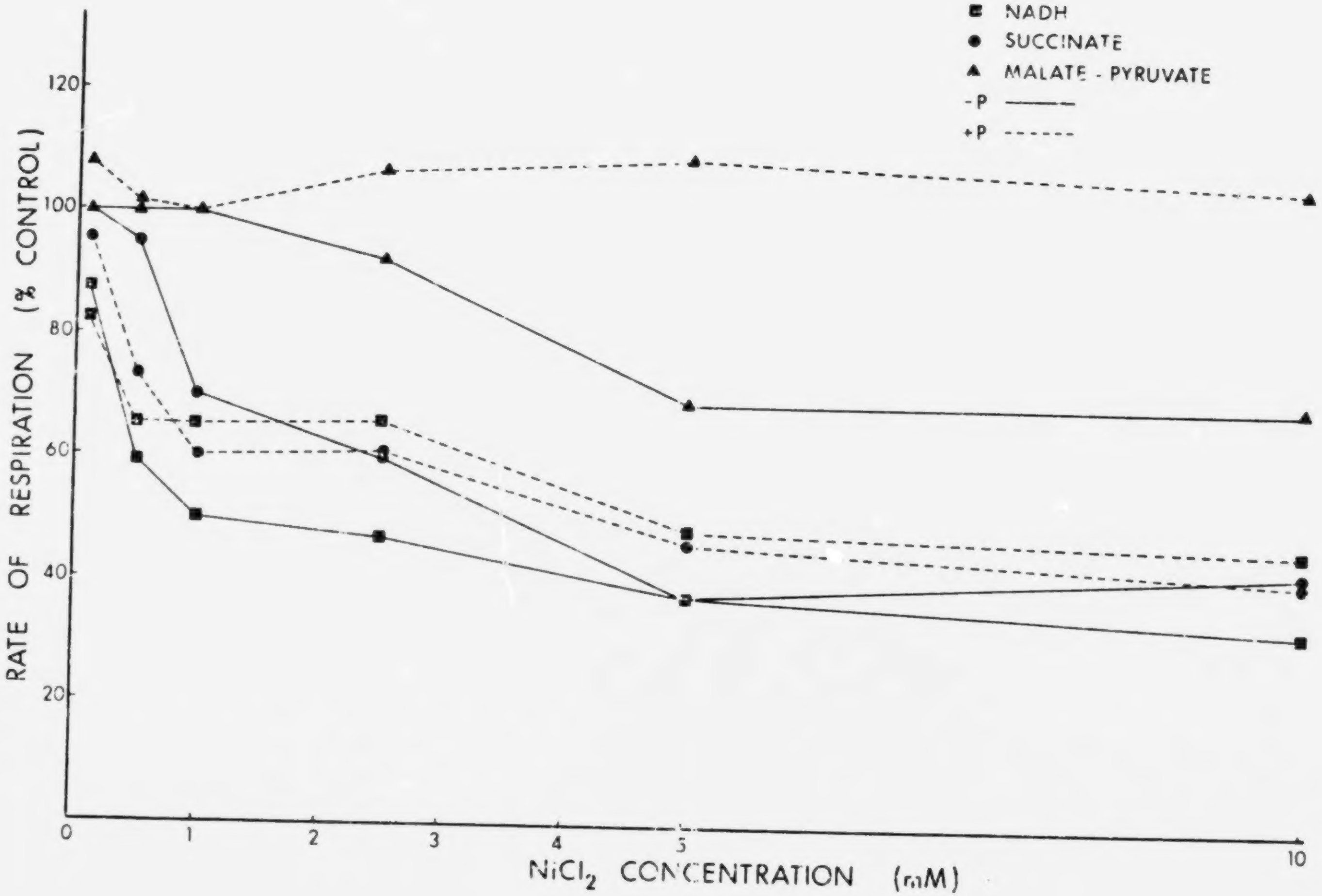

Figure 82. The effects of phosphate on the NiCl$_2$-induced changes in rates of O_2 uptake. See Figure 66 for experimental details of -P studies and Figure 67 for +P studies.

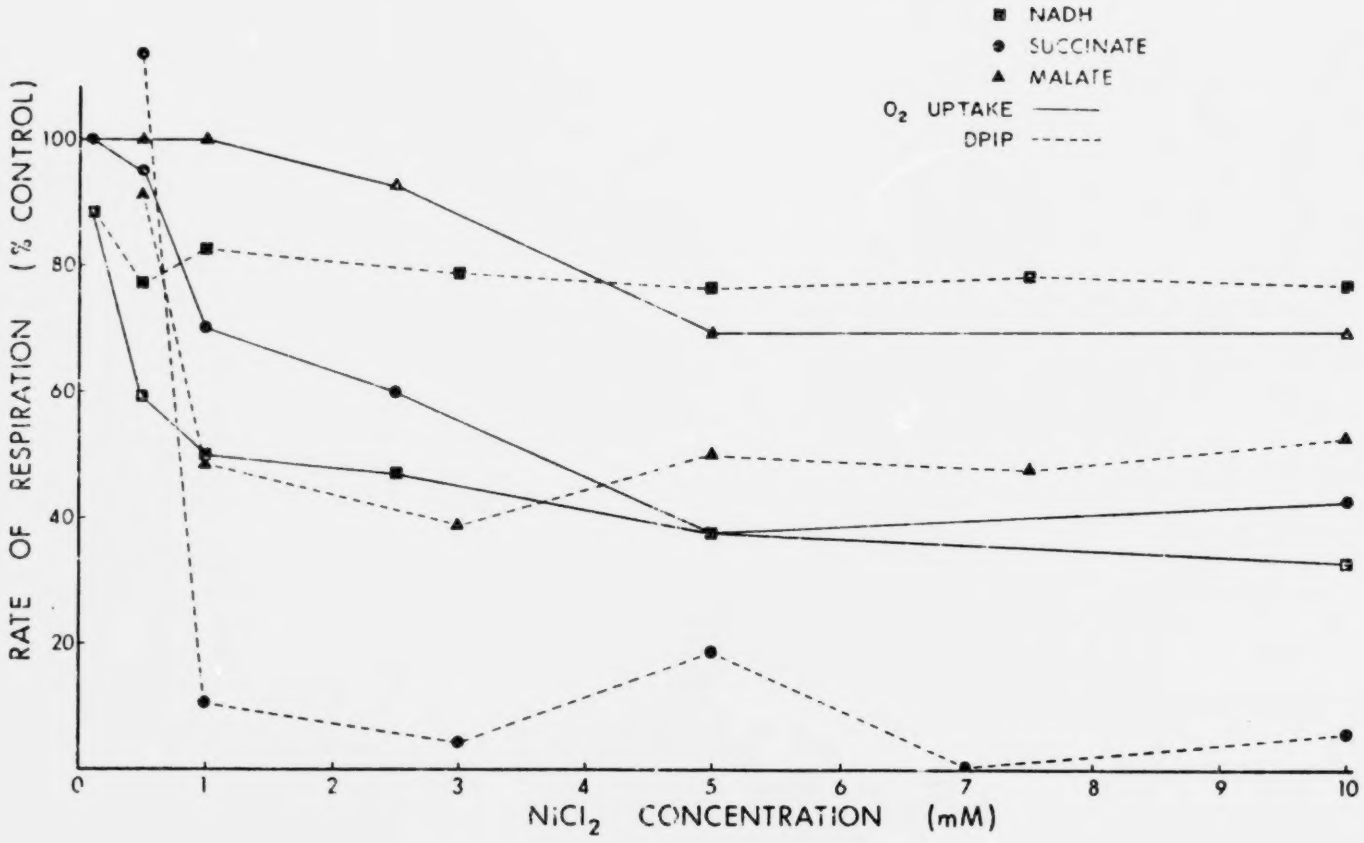

Figure 83. A comparison of the effects of $NiCl_2$ on the rate of electron flow from substrate to oxygen and from substrate to 2,6-dichlorophenolyindophenol. See Figure 78 for details.

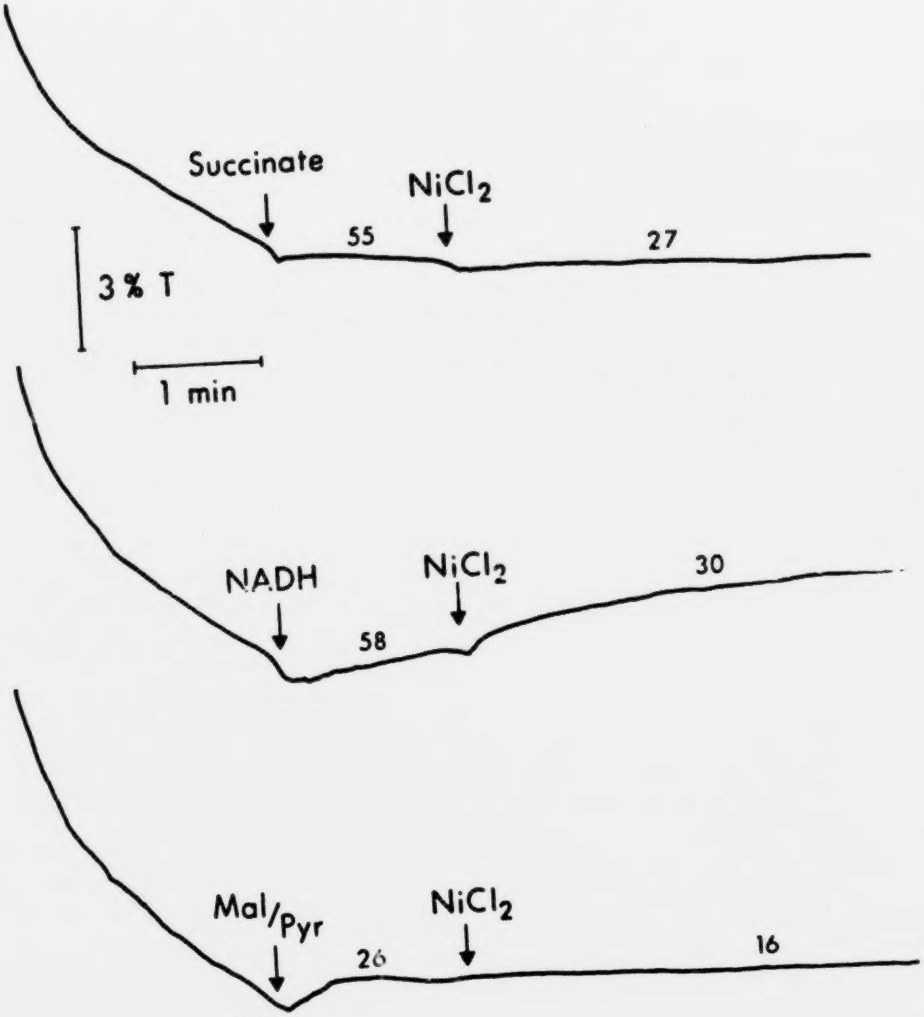

Figure 84. The effects of $NiCl_2$ on swelling of actively respiring mitochondria. Traces represent percent transmittance of light with swelling being a downward deflection. Numbers next to traces represent concomitant rates of O_2 uptake expressed of nmoles O_2/min x mg mitochondrial protein. Additions of 40 μmoles succinate, 2 μmoles NADH, or 40 μmoles malate + 40 μmoles pyruvate were made as indicated. Concentrations of $NiCl_2$ after additions were 1 mM for succinate, 1 mM for NADH, and 5 mM for malate + pyruvate.

3. Energy coupling

Nickel reduced both ADP/O and respiratory control ratios in a manner analogous to that previously reported for Cd, but at comparably higher concentrations to those encountered for Ni-induced swelling (Figures 85 and 86). Phosphorylation associated with malate + pyruvate oxidation was less affected at comparable concentrations than was that associated with succinate or exogenous NADH oxidation.

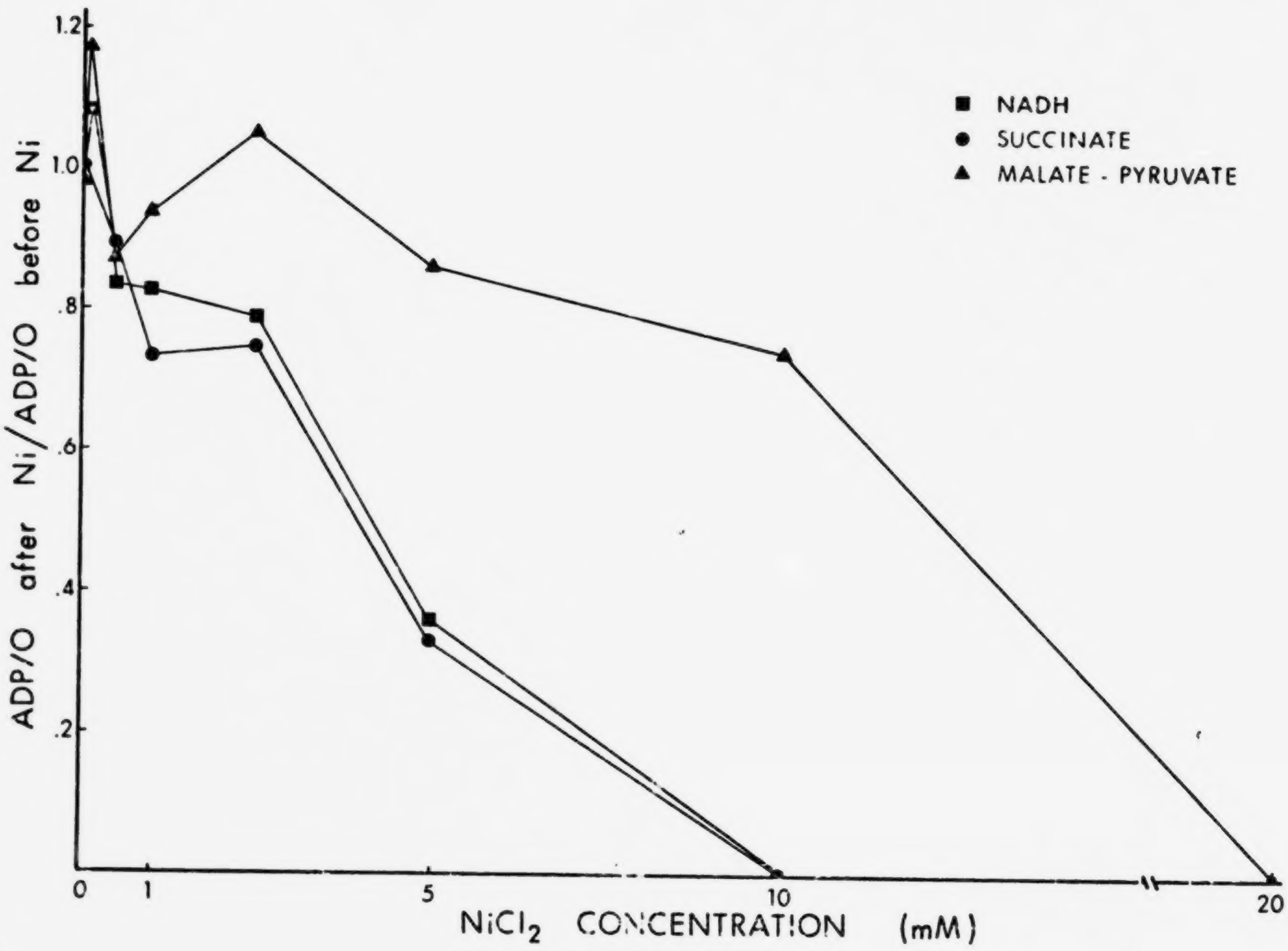

Figure 85. The effect of varying $NiCl_2$ concentrations on mitochondrial coupling expressed as ADP/O ratios. See Figure 75 for details.

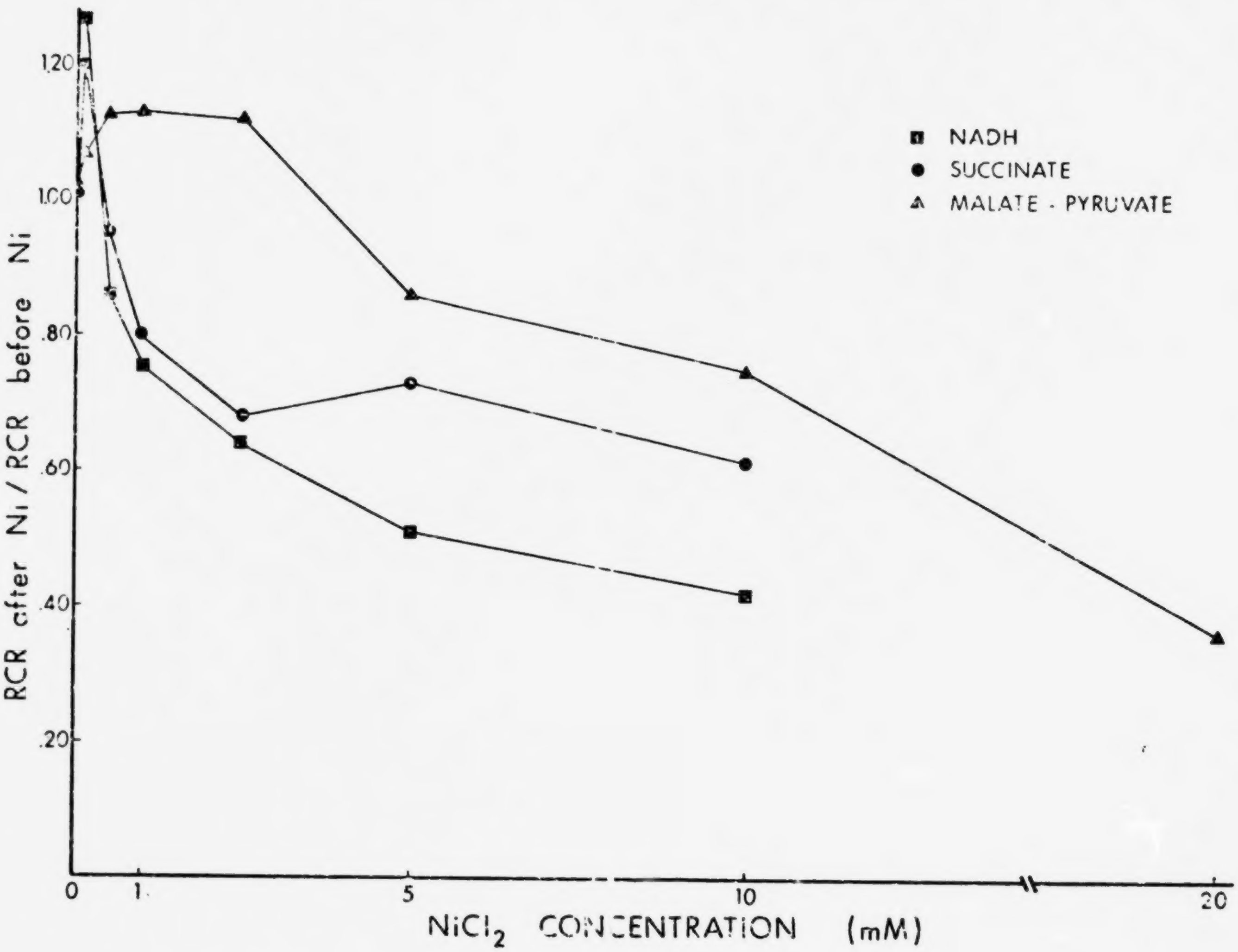

Figure 86. The effect of varying $NiCl_2$ concentrations on mitochondrial coupling expressed as respiratory control ratios. See Figure 75 for details.

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VII. ANIMAL PROCESSES

INTRODUCTION

The work done by the Animal Processes Group has centered on three main areas of concern in metabolism of lead by animals. One of these areas involves interaction of lead with membranes, since it has been postulated that the leakage of K^+ from red blood cells treated with lead either in vivo or in vitro (Passow, 1961) is a result of lead interaction with the cell membrane. Since the red blood cell is a target organ for lead (Chisolm, 1971) and its membrane is less complex than that of other tissues, this organ may well serve as a model for the interaction of lead with other cell systems. Furthermore, the lipid extracts of these cells can be obtained and made into model membranes, i.e., vesicular dispersions of the lipid in water and bilayer lipid membranes (Chapman, 1960). With these model systems, physico-chemical types of measurements can be made; the measurements include permeability, electrical conductivity, and nuclear magnetic resonance (NMR) studies of the membranes.

Lead affects the RBC in a variety of ways, the end result being impairment of the RBC function. A K^+ leakage from inside to outside of the cell is one of the most notable effects of lead, both in vivo and in vitro (Passow, 1961). The origin of this effect is presently unexplained and has been postulated to result from the interaction of lead with the cell membrane. A corollary of this hypothesis is that the lead interacts with some lipid component in the membrane. Other workers (Barltrop, 1971), however, maintain that very little lead is actually bound to the membrane but rather almost all of the lead is bound to cell proteins. One of the difficulties in studying this effect is that most of the lead that is thought to bind to the membrane may bind non-specifically and therefore is not responsible for the biological damage. In addition to the possible damage to the membrane, lead does enter the immature RBC and there interferes with heme synthesis. In order to enter the cell, lead, in some form, must first be adsorbed to the membrane surface; this lead is probably not the lead that causes the K^+ leakage. In all cases, little is known of the actual chemical form in which the lead interacts with the cell.

The second area of research attacks a major physiological consequence of lead toxicity, anemia resulting from defective synthesis of the heme portion of hemoglobin in the immature red blood cell. *In vitro* heme synthesis may conveniently be studied as an overall process, or portions of the synthetic pathway may also be investigated during development of lead toxicity. In this latter area a known aberration in amino levulinic acid metabolism is recognized in lead toxic animals and for this reason was investigated in these studies. The third general area of experimentation relates to the rate and extent to which lead is accumulated by various tissues of animals fed different amounts of lead and to the removal of lead from these tissues after the lead exposure has ceased. This information is conveniently obtained with laboratory animals such as the albino rat and can serve as a model for the study of lead flow through a mammalian system. These three areas of research are treated separately in the following presentation.

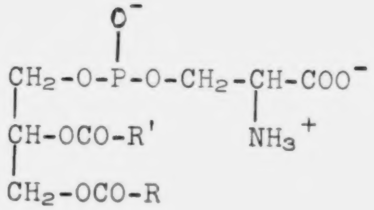
A. Membrane-Lead Interactions

1. Methods

In the work of this report, the membrane is being studied in three different physical forms: the sonicated fragments of human RBC's; sonicated dispersions, in water, of vesicles formed from the lipid extracts of the RBC's and from pure phospholipids (Figure 87); bilayer lipid membranes formed from the lipid extracts of the RBC's. The first two model systems are amenable to spectroscopic measurements such as nuclear magnetic resonance (NMR) measurements. The bilayer lipid membrane is more suitable for permeability and electrical conductivity measurements. In all cases, studies of these model systems with respect to their interaction with lead, should provide information pertinent to the effect of lead on RBC's and other cell systems and the transport of lead across the cell membrane.

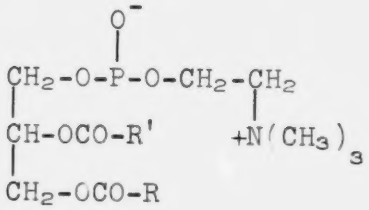
2. Results

Bilayer lipid membranes (BLM) were initially formed from oxidized cholesterol (Tien, 1966). These membranes provide a stable system for calibration and comparison purposes. Cholesterol, moreover, is an important constituent of the RBC membrane. In the presence of 10^{-2}M $PbCl_2$, these membranes exhibit a significant lowering of their electrical resistance, with time. This effect is shown in Figure 88. The effect does depend on the type of oxidized cholesterol used. Cd^{++}, moreover, has a much smaller effect on the electrical conductivity than does Pb^{++}. Nernst potential measurements are now being made in order to get some idea of the lead species which is responsible for the increase in electrical conductivity.



$$\begin{array}{l}
\qquad\quad\;\; \mathrm{O^-} \\
\qquad\quad\;\; | \\
\mathrm{CH_2-O-P-O-CH_2-CH-COO^-} \\
| \qquad\qquad\qquad\qquad\quad | \\
\mathrm{CH-OCO-R'} \qquad\;\; \mathrm{NH_3^+} \\
| \\
\mathrm{CH_2-OCO-R}
\end{array}$$

Phosphatidyl Serine (PS)



$$\begin{array}{l}
\qquad\quad\;\; \mathrm{O^-} \\
\qquad\quad\;\; | \\
\mathrm{CH_2-O-P-O-CH_2-CH_2} \\
| \qquad\qquad\qquad\qquad\quad | \\
\mathrm{CH-OCO-R'} \qquad {+}\mathrm{N(CH_3)_3} \\
| \\
\mathrm{CH_2-OCO-R}
\end{array}$$

Phosphatidyl Choline (PC)

Figure 87. Phospholipid Molecules.

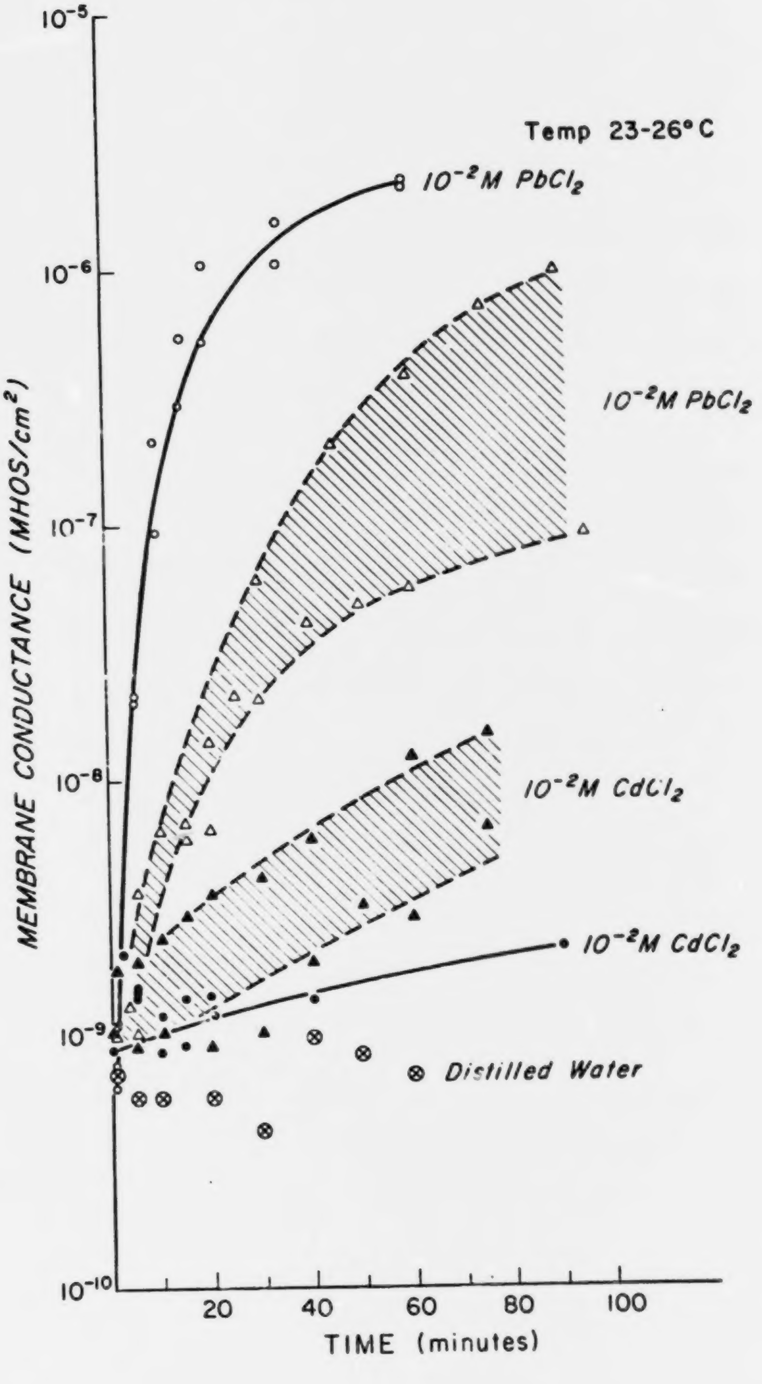


Figure 88. Membrane Conductance of
Oxidized Cholesterol I
Oxidized Cholesterol II.

BLM formed from the RBC lipid extract, on the other hand, exhibit little if any change in their electrical conductivity upon exposure to 10^{-2}M $PbCl_2$. This is true both in the presence of a potassium phosphate buffer as well as in its absence. KCl, in the presence or absence of lead, also has no effect on the electrical conductivity.

In the presence of a tenfold concentration gradient of $PbCl_2$, across the RBC lipid BLM, a Nernst potential of approximately 30 mv is obtained. This is shown in Figure 89. A tenfold gradient of KCl produces no Nernst potential, in the presence or in the absence of $PbCl_2$. The sign and magnitude of the Nernst potential suggests that Pb^{++} is the species crossing the membrane.

NMR spectra of both sonicated RBC ghost fragments and sonicated dispersions (Jendrasiak, 1972) of the lipid extracts of the ghosts were obtained. In the presence of $PbCl_2$, the spectrum of RBC ghost fragments is, apparently, changed from the case with no $PbCl_2$ present, whereas that for the lipid extracts is not. These results are shown in Figures 90 and 91. In the case of the RBC fragments, the signal due to the $N^+(CH_3)_3$ polar head group of the lipids is shifted by the lead. A similar shifting occurs for the N-acetyl proton signal from neuraminic acid. The rest of the spectrum is apparently unaffected by the lead. The spectra of the lipid extracts are apparently the same with and without the presence of lead. The effect on the NMR signal of the RBC fragments due to lead is, at present, not reproducible. The problem of reproducibility is presently being studied, especially with respect to the sonication procedure. With the small amount of data presently available and with the lack of reproducibility, it is difficult to draw any firm conclusions.

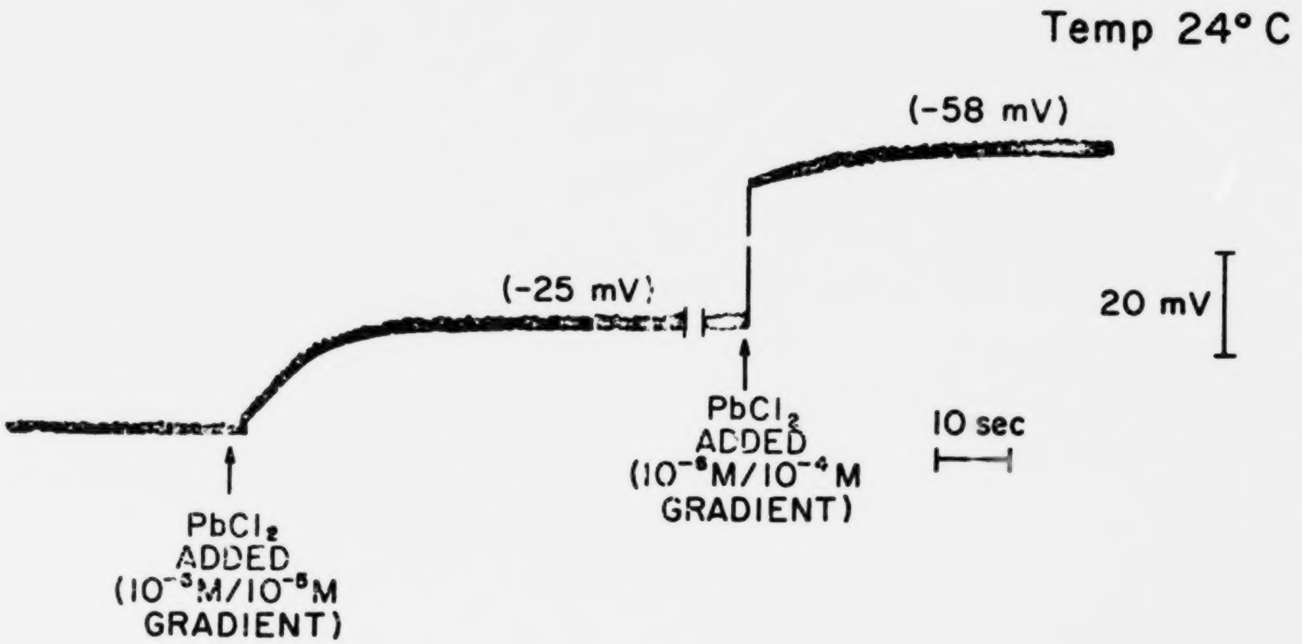


Figure 89. Nernst Potential, RBC Bilayer Lipid Membrane.

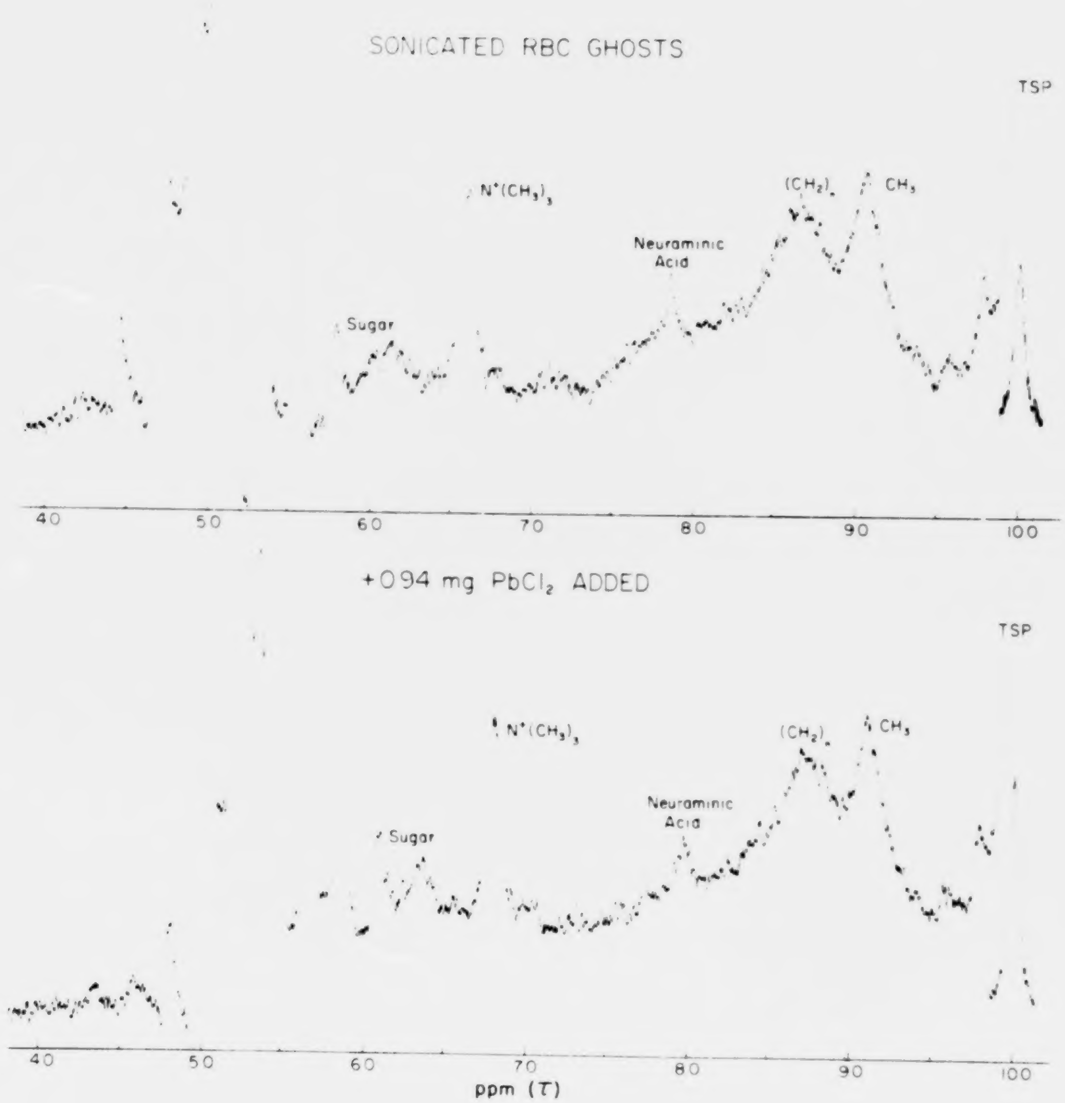
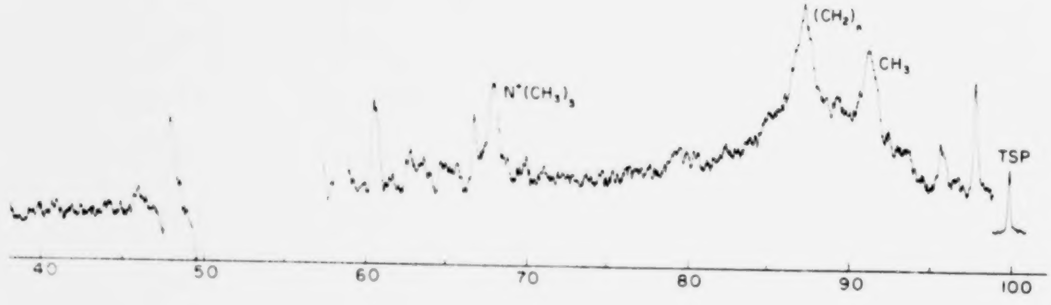


Figure 90. NMR Spectra of RBC Membrane Fragments in D_2O.

SONICATED RBC LIPID



+24 mg $PbCl_2$ ADDED

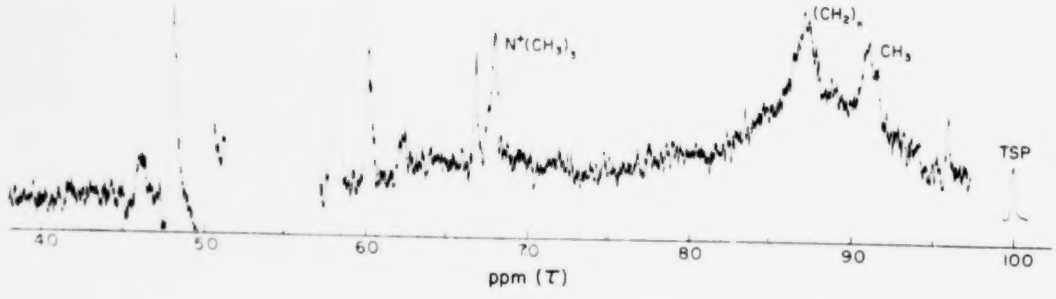


Figure 91. NMR Spectra of RBC Lipid Extract in D_2O.

On the basis of the Nernst potential measurements, Pb^{++} can cross BLM formed from RBC lipids. Pb^{++}, on the other hand, has no effect on the K^+ permeability of the BLM. It must be remembered, of course, that the Model membrane is, in all probability, structurally quite different from the RBC membrane itself. Also, further work will tell whether Pb^{++} crosses BLM to any greater degree than do other divalent metals.

The effect of $PbCl_2$ on the electrical conductance of BLM formed from oxidized cholesterol is of some interest. Cholesterol has been postulated as being a possible complexing agent for Pb^{++} in RBC's (Passow, 1961). The effect of $PbCl_2$ on the electrical conductivity of the BLM is certainly consistent with such an interpretation. Since BLM formed from the lipid extract of RBC contains almost 50% cholesterol, however, it is difficult to see why a similar effect does not occur for the electrical conductivity of RBC-BLM. It may be that the state of cholesterol in RBC-BLM is quite different from that in oxidized cholesterol-BLM. The biological implications of this remain to be elucidated.

The NMR techniques should provide a surer method of looking at the lead-membrane interaction. Here the pure lipids can be compared with the total membrane fraction. The data are sparse at this point but there does seem to be an effect of lead on the total membrane fraction, as determined by NMR, whereas there is no effect of lead on the lipid extract. Since the presence of lead results in a shift of the polar head group signal as well as that of the N-acetyl proton signal, several possibilities arise: 1) the lead could interact with both the polar head group and with the N-acetyl group (the interaction strength might be quite different in the two cases); 2) the lead could be interacting with one or the other of these sites and the resultant change in membrane conformation could be responsible for the change in NMR signal from the other site. Other possibilities are also quite possible. At present, these various possibilities cannot be distinguished. Indeed, none of these possibilities need be related to the biological effects of lead on RBC, but this remains to be seen.

One phase of our research was designed to characterize the phospholipid molecules in vesicles by NMR spectroscopy. Spectra of both PC and PS were assigned and it was noted that the majority of resonance lines indicated the superposition of a narrow line on a somewhat broader line. This indicates the presence of at least two molecular environments in a vesicle. Using the paramagnetic broadening properties of Mn^{++}, it was shown the Mn^{++} could get close to a little more than half the molecules in the vesicle but not to the rest. When the sample was heated to 70°C. the Mn^{++} was able to approach all molecules and this behavior persisted when the sample returned to 39°C.

This behavior is interpreted to mean that the vesicles consist predominately of bilayers [not as multilayers as suggested by some workers (Dervichian, 1964)]. The Mn^{++} can thus approach closely only to the outer bilayer since the inner is situated in the central part of the spherical structure. Heating the vesicles allowed the Mn^{++} to enter the structure and get close to the molecules in the inner layer. This explanation accounts for the observation that the NMR resonance peaks are composite, the narrow lines being from more mobile molecules in the inner layer. Similar results have been reported concurrently by some Russian workers (V. F. Bystrov, et al., 1971) and the results of studies of anion-vesicle interactions by Jendrasiak (1972) point to the same conclusion.

Some preliminary results based on the relaxation times of nuclei at a given distance from a paramagnetic species (H. S. Gutowsky, et al.,

1972) (Mn^{++}) indicate that the phospholipid molecules in a bilayer have the conformation of their hydrophilic chains as shown in Figure 92a rather than that shown in Figure 92b. Both have been proposed in the literature.

Observation of the NMR spectra of PS and PC on addition of metal ions (as the chloride or nitrate) shows that the following ions interact with PS so as to produce spectral broadening. This is interpreted in terms of reduced mobility of molecules in a vesicle or of the vesicles as a whole. In some cases actual formation of a precipitate has been observed. The evidence is clear, therefore, that an interaction of some sort occurs with PS. No such behavior is observed with PC. In approximate order of increasing effect on the NMR spectra the following ions interact with PS:

Na^+, K^+, Ag^+, Mg^{++}, Cu^{++}, Cd^{++}, Ca^{++}, Pb^{++}, Cr^{+++}, and La^{+++}.

This series shows a rough correlation between effect on the NMR spectra and both the charge and size of the cation. Although the effects of these metal ions on the NMR spectra of PC were very slight, there is marginal evidence that some interaction does occur.

The simplest explanation of these results is that the cations form a simple complex with individual negatively-charged phosphate or carboxyl groups in the lipid or a chelate complex between adjacent groups in the same molecule or in neighboring molecules in the vesicle. This would explain why introduction of metal ions restricts the mobility of the molecules in the vesicle. One might expect PC not to be capable of such complex formation because of the extra size of its terminal or "head" group $N(CH_3)_3^+$ and its lack of a negatively-charged carboxyl group.

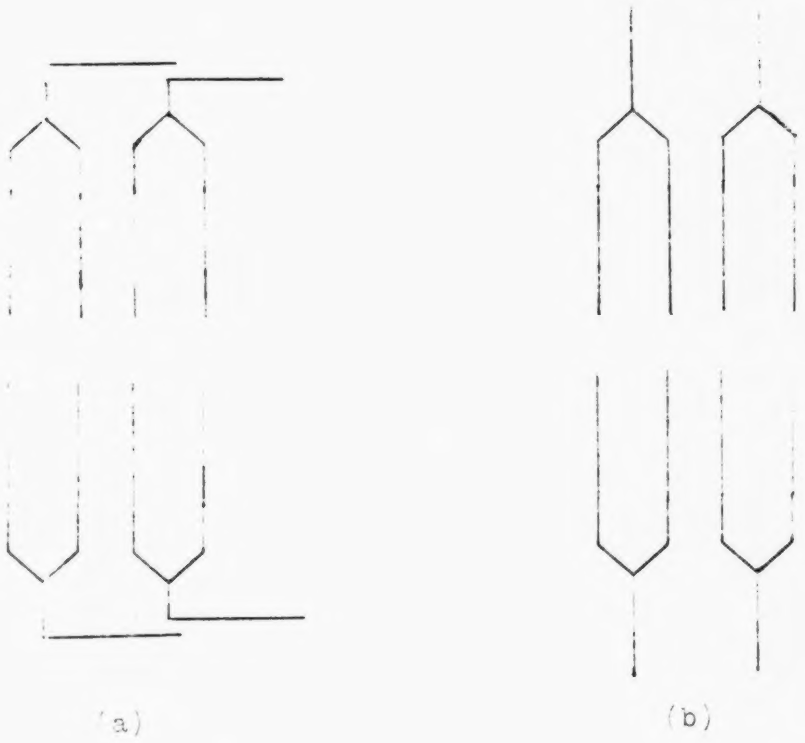


Figure 92. Possible Conformations of Phospholipids in a Vesicle.

Marginal evidence for such complex formation has been obtained from the effect of metal ions on the ionization behavior of PS. Thus, ionization occurs at a slightly lower pH in the presence of metal ions --notably, Ca^{++} (M. B. Abramson, et al., 1966; N. M. Miller, 1971). This result, however, is open to many interpretations as the vesicle structure definitely changes with pH.

If conventional metal-ligand complex formation occurs between metal ions and phosphate groups, it is almost axiomatic that there should be some change in the electron distribution in the molecular orbitals close to the binding site of the metal. Such a rearrangement, in all known cases, results in a change in the NMR chemical shift of the nuclei close to the binding site (D. F. S. Natusch, et al., 1971). However, we have observed no changes in any proton chemical shifts in either PC or PS on addition of metal ions. This might be possible in the case of protons since the closest proton to the phosphate group is 4 bond lengths away from the metal-phosphate linkage. However, ^{31}P NMR of the phosphorus itself also shows no change in chemical shift.

The inescapable conclusion is that although metal ions including lead definitely associate with PS they definitely do not form conventional metal-ligand bonds. This conclusion was remarkably well demonstrated in some PC and PS samples which had been disposed with high power sonication where localized heating had occurred. The ^{31}P spectra of these samples showed distinct resonances due to phosphate groups either in individual molecules not present in vesicles or to fragments of degraded phospholipids. On addition of Ca^{++}, Cd^{++}, or Pb^{++}, these phosphate resonances moved ∿400 Hz upfield indicating beyond question that the phosphate group was complexing with the metal ions (N. M. Miller, 1971).

Thus, phospholipid molecules present in vesicles do not form conventional complexes with Pb^{++} and other cations, but those not present in vesicles do.

Jendrasiak (1972) has shown that small interactions between anions and the $N(CH_3)_3^+$ group of PC do occur for large polarized anions such as I^-, I_3^-, and SCN^- giving rise to small $N(CH_3)_3^+$ chemical shifts but again the actual amount of electron density disruption is small.

Clearly, therefore, molecules present in a vesicle behave very differently from individual molecules although the vesicles (of PS at least) are definitely capable of binding cations. At present, our interpretation is that the cations are bound not to phosphate groups per se but rather to the electrical double layer (or Stern layer) which surrounds the surface of the vesicles. Using paramagnetic Mn^{++} as a probe to determine the distance of closest approach of a cation to individual molecules in the vesicle indicates that binding to an "outer surface" is distinctly possible. Thus, the distance of closest approach of Mn^{++} to phosphate is too great for conventional bond formation.

Jendrasiak's work (1972) with anions suggests that metal binding to vesicles may depend upon the accompanying anion and work with Mn^{++} halides bears out this suggestion.

Thus, Pb^{++} appears to behave essentially similarly to other cations in that it enters the electrical double layer surrounding phospholipid vesicles. The strength of binding appears to depend on the electrical charge, size, and counteranion of the metal ion. These suggestions are being investigated further. particularly with respect to organic Pb compounds.

On the basis of the results with PS, we would expect the presence of a small amount of Pb^{++} (and many other cations) to markedly affect the conductance properties of PS bilayer membranes. This has been confirmed by Jendrasiak (1972), thereby demonstrationg the worth of structural information in elucidating membrane behavior.

Despite considerable effort, the apparently simple experiment of monitoring Pb^{++} uptake by phospholipids with ion-selective electrodes has proved unsuccessful. Both Pb^{++} and Cd^{++} ion-selective electrodes indicate an increase in cation concentration when PS is added to the cation solution. This is highly improbable and is almost certainly due to interaction of the phospholipid with the sensitive detecting element of the electrode so as to create a junction potential. When the electrode is shielded with a dialysis membrane through which Pb^{++} can pass, but not the phospholipid, similar results are obtained. It is understood that similar problems have been encountered by other workers and are at present unsolved.

Clearly, we will have to rely on NMR as a screening tool to determine whether different phospholipid vesicles are capable of binding lead. This is definitely advantageous where single phospholipids are concerned. However, in screening human blood extracts or model membranes containing more than one component (e.g., other phospholipids, cholesterol, or proteins), the complexity of the proton NMR spectra will rapidly increase so as to provide minimal information. It is intended, therefore, to undertake studies with ^{13}C NMR which provides much greater distinction of individual nuclei than does proton NMR.

Further work will concentrate on obtaining more information about the electrical double-layer binding, on factors which affect the transport of Pb^{++} across the model membranes. Particular emphasis will be placed on red blood membranes in various degrees of refinement.

B. Studies Related to Heme Synthesis

1. Methods

a. Dietary vitamin effects on heme synthesis. Three experiments were conducted according to the following general plan. Important deviations from the plan are noted in the section on results. Young albino rats were fed none or 300 ppm lead added as lead acetate in a 20% casein, corn oil, glucose diet designed to be adequate but not excessive in minerals and vitamins. The feeding period lasted six weeks during the last two of which all animals received a daily subcutaneous injection of phenylhydrazine. The purpose of the latter was to cause hemolysis of mature red blood cells and thus stimulate reticulocytosis, since it is in the nucleated reticulocytes that heme synthesis occurs and these are small in number in the normal rat. This treatment increased their numbers tenfold. Twenty-four hours before killing, the animals were given selected vitamin treatments intraperitoneally. Finally blood samples were obtained from abdominal aorta and these were incubated for study of heme synthesis. An appropriate medium containing glycine-2-C^{14} was used and incubation was for 12 hours, aerobic. Following incubation, heme was isolated, purified and radioactivity counted by scintillation counting. Since the rigorous purification steps required yielded only about 50-60% of the heme, total heme analysis before and after incubation was also made by the the cyanohematin method.

b. Effects of lead dosage on amino levulinic acid (ALA) metabolism. This study was made to determine the effect of graded single doses of lead on urinary excretion of ALA and on the activity in several tissues of ALA synthetase and ALA dehydrase, the enzymes directly involved in synthesis and subsequent metabolism of ALA.

On the basis of our earlier findings that urine ALA of lead-poisoned animals rose sharply and then decreased with time, an experiment was undertaken to determine whether these changes were a result of increased production or increased utilization of ALA. Young male rats were given basal purified diet for three days and then fasted 24 hours. They were then given 2 gm of diet containing 0, 100, 250, 500, 1500 or 3000 gamma of lead (equivalent to 10-300 ppm in diet of normal 10 gm daily intake). They cleaned this allotment up within two hours and then were given the basal lead-free diet ad lib for the remainder of the experiment. Animals were killed at planned intervals and blood, liver, kidney and urine were obtained for analysis of ALA and its forming and metabolizing enzymes, ALAS and ALAD. For ALAS analysis, tissue homogenates of lysed whole blood were incubated with glycine and glucose as substrates, the ALA formed was converted to a pyrrole with heat and acetylacetone and the pyrrole reacted with Erlich's reagent to give a red color (ALAD activity was blocked with EDTA). ALAD activity was measured, using ALA as substrate. The porphobilinogen formed is a pyrrole which also reacts with Erlich's reagent.

c. Effects of lead on tryptophan pyrrolase. Along another line, since hemoglobin is a slowly turning-over compound, we thought of looking at synthesis of a more rapidly metabolized heme enzyme on the basis that a lead-induced defect in its synthesis might be more easily demonstrated. For this purpose we chose tryptophan pyrrolase, with a turn-over rate of 2-3 hours, and also since it is influential in synthesis of niacin from tryptophan.

Young chicks were fed a purified amino acid diet for 2 weeks with 0, 50, 100 or 200 ppm lead added. At the end of this time liver and blood were obtained.

2. Results

a. Dietary vitamin effects on heme synthesis. The lead treatment inhibited weight gain by about 20% by week 4, and blood heme (tail sample) at this time was reduced by 28% in the first experiment. Table summarizes the heme synthesis data of the first experiment.

This experiment was also essentially repeated without lead treatment to verify that the effects seen were not a result of the phenyhydrazine treatment. No responses to vitamin supplementation were seen in these animals.

These experiments indicated that niacin and a combination of vitamins C and B_{12} were partially effective in stimulating heme synthesis in reticulocytes of lead-treated animals. We attempted to investigate this further in a 2^3 factorial design involving single and combined treatments with these three vitamins. The experiment was largely a failure but worthy of reporting, since it illustrates a lesson. In this experiment the rats were given 300 ppm lead as in the preceding one, but they appeared to react much more severly so that the animals were given phenyhydrazine at day 21 instead of 28 and for only five days, at which time several of them had died and the remainder did not appear well. When blood samples were obtained they appeared to be dark brown instead of red, indicating a large degree of hemolysis by the drug. Nevertheless, heme analyses and incubations were made but no treatment

Table 38

Blood Incubation Study (Experiment 1).

Treatment	Total DPM[1]	Heme mg/4 ml blood	
		Before Incubation	After Incubation
No lead	5302 **	16.4	19.9
Lead			
Saline	2159	8.7	12.9
3 mg B_6	3177	8.7	11.1
30 mg niacin	3653 *	10.6	15.1
2.5 mg B_1 + 5 mg B_2	2848	10.9	12.7
10 mg C + 0.01 mg B_{12}	3724 *	8.7	15.6

[1] Disintegrations per minutes

* Significantly different (CL= 95%) from saline control

** Significantly different (CL= 99%) from saline control effects (vitamin) were seen. Blood heme was 6-8 mg/4 ml as compared to 8-10 in the first experiment. It turned out that the diets used in this experiment contained only about 0.3% calcium instead of the expected 0.6%. The fault lay in the commercially obtained mineral mix with which we have occasionally had problems before but did not have the wit to check this time. It is well documented that calcium deficiency enhances lead toxicity.

In the third experiment we collected urine every three days and analyzed the samples for aminolevulinic acid with results as shown in Table 39. We were intrigued by the initial high ALA and its decrease, so the next experiment was designed with the twofold purpose of investigating the previously observed niacin effect in a different manner and obtaining more detailed data on ALA excretion as affected by lead. Normal and 10 X normal levels of niacin were used in the diet throughout and the animals were fed 0, 50, 150 and 300 ppm lead for three weeks and then were given phenylhydrazine for one week. Urine was collected daily and analyzed for ALA. At the end of the experiment total heme was analyzed in blood before and after incubation.

Although all supplementary lead levels increased urinary ALA, the response was associated with lead level and not with niacin, as seen in Figure 93. Again the fact of an initial peak followed by a decrease was observed. The heme assay gave results as seen in Table 40.

At week 4, tail blood samples were taken for heme analysis, and at this time no lead effect could be seen. The samples taken for incubation were taken after phenylhydrazine treatment and the progressive lowering of heme seen is probably a result of the increased sensitivity of lead-treated red cells to pherylhydrazine, an effect not dependent on niacin supply. The increased heme synthesis seen in the blood of animals receiving

Table 39

Urinary ALA (Experiment 3)

Days of Lead Treatment	µg ALA/mg creatinine
6	330
9	200
12	200
15	210
18	200
24	175

Table 40

Heme Synthesis (Experiment 4)
(mg Heme/4 ml Blood)

ppm Pb	0	50	150	300
Normal niacin				
Before incubation	14.0	12.7	11.4	11.0
After incubation	15.9	14.4	12.8	12.8
Synthesized	1.9	1.7	1.4	1.8
High Niacin				
Before incubation	14.6	13.6	12.3	11.1
After incubation	16.5	15.6	14.9	13.5
Synthesized	1.9	2.0	2.6	2.5

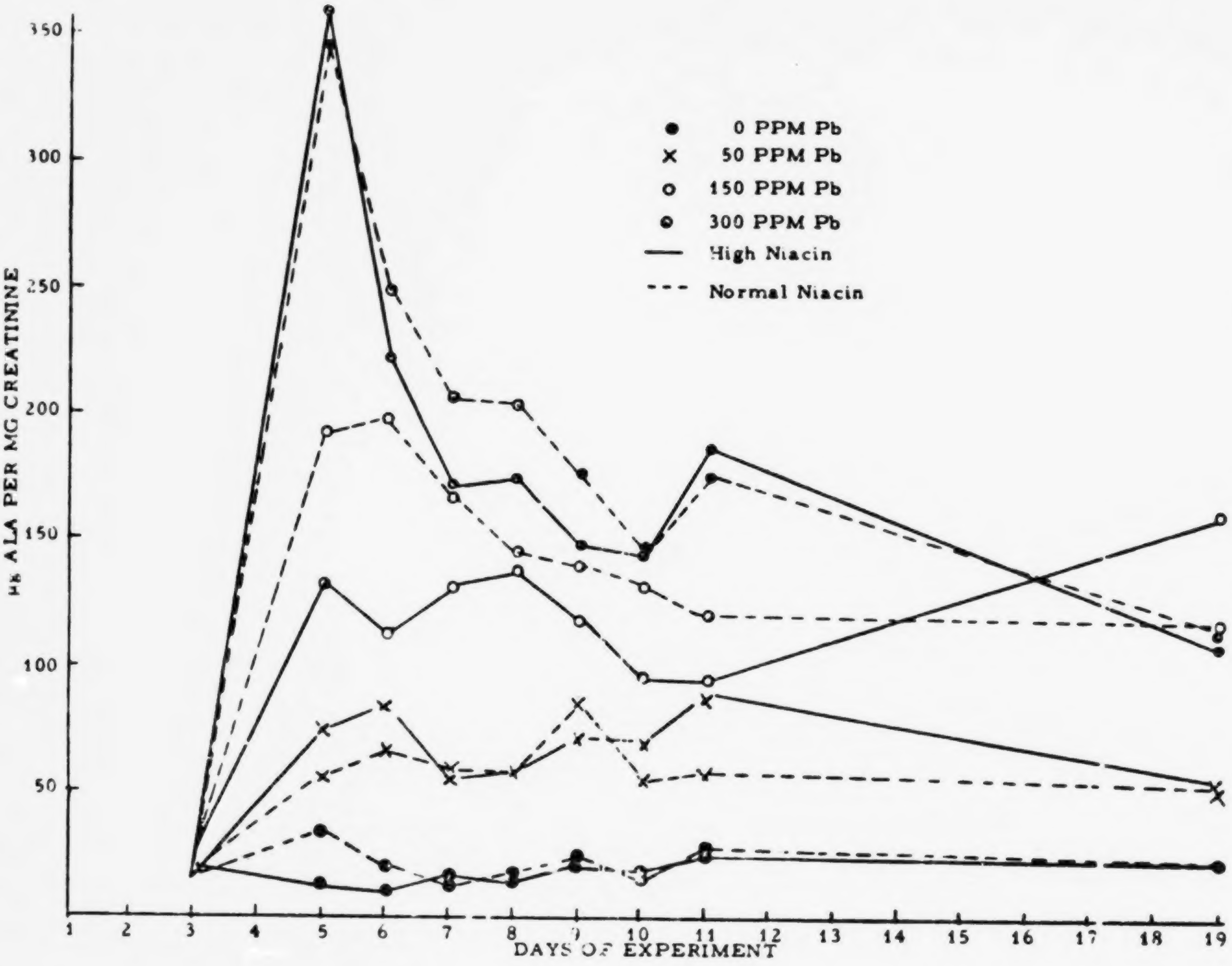


Figure 93. Average Urinary ALA Excretion

high niacin and high lead is probably a result of stimulation of reticulocytosis by phenylhydrazine in the high-lead animals and the ability of niacin to permit a response in terms of heme synthesis. In other words, there were more reticulocytes in the high-lead animals but they could respond in terms of heme synthesis in the presence of lead only under the influence of superabundant niacin.

b. Effects of lead dosage on ALA metabolism. As seen in Figure 94, urine ALA was not affected by the two lowest levels of lead treatment but was increased by the three highest at 12 hours after treatment. Elevated values that were somewhat dose-dependent peaked at 3-4 days and then declined. These effects followed a single exposure to lead.

The next series of Figures, 95 through 100, shows the time-relationships of ALAD and ALAS in blood, liver and kidney. ALAD activity in blood and kidney was maximally decreased at six hours and returned gradually to normal. In liver the ALAD response was notably only in rats receiving the two highest doses of lead. An initial decrease was followed by a persistent overshoot starting at 48 hours, probably a result of stimulation of enzyme activity by increased substrate (ALA) in the tissues.

ALAS analyses are not so reliable since blanks are high and activity usually lower than ALAD blanks, thus measured activity was more variable but was definitely elevated at 12 hours in blood and at 24 hours in liver and kidney at the higher levels of lead. The earlier response of ALAD than ALAS is probably because the former is a cytoplasmic enzyme and the latter a mitochondrial one and more time is required for the lead to enter the mitochondria. Early response is in blood because this is where lead first accumulates.

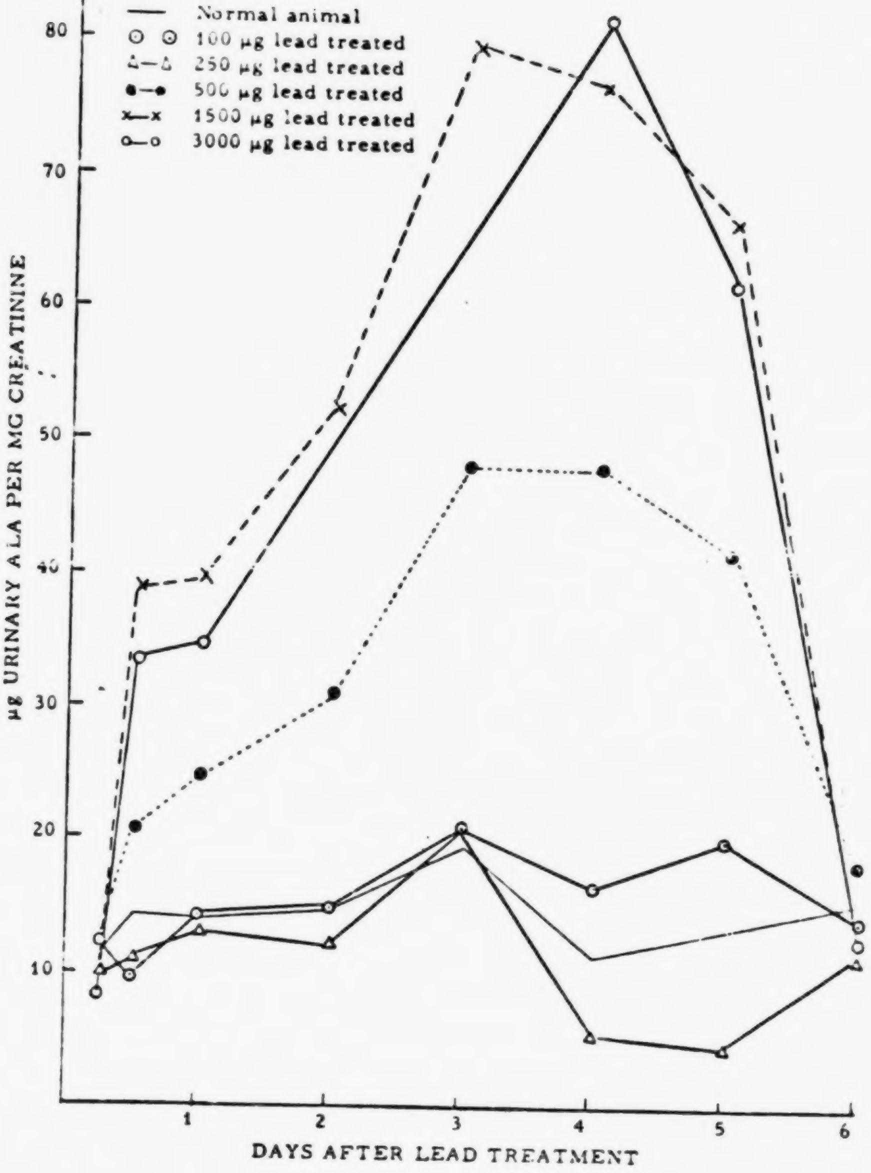


Figure 94 Effect of Lead on Urinary ALA Excretion.

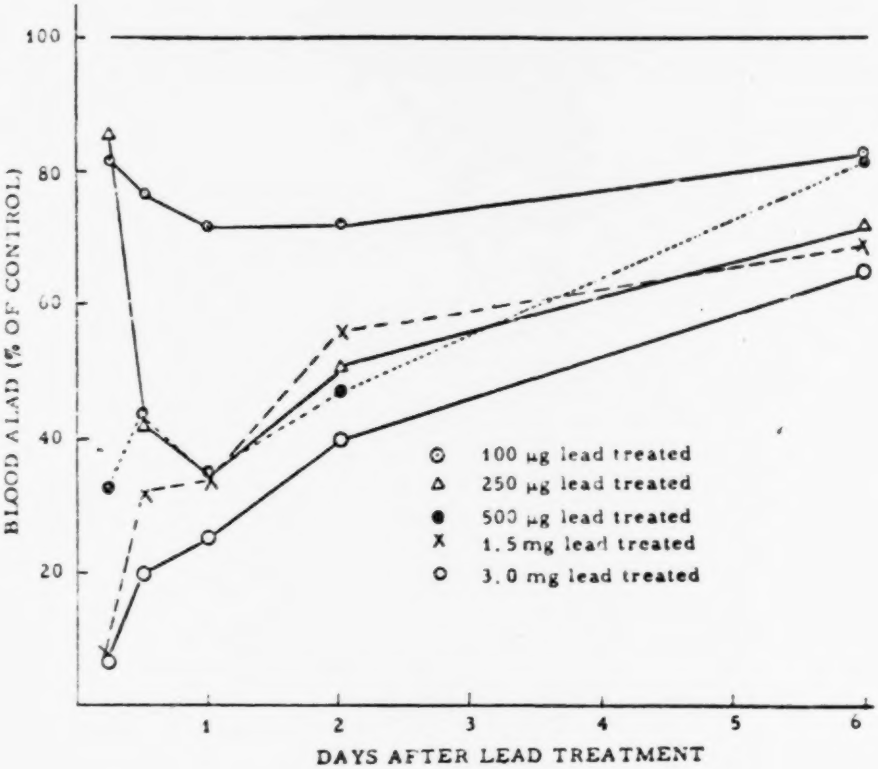


Figure 95. Effect of Lead on Blood ALAD Activity

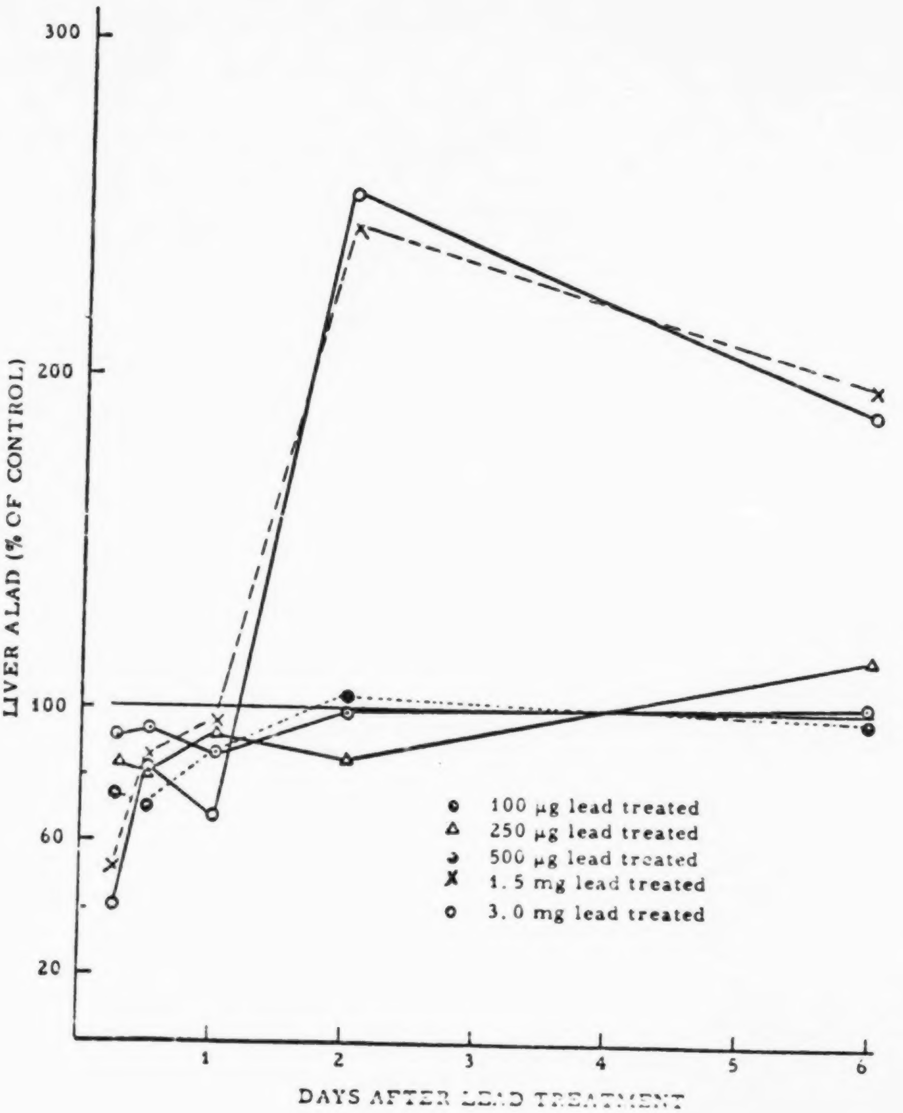


Figure 96. Effect of Lead on Liver ALAD Activity.

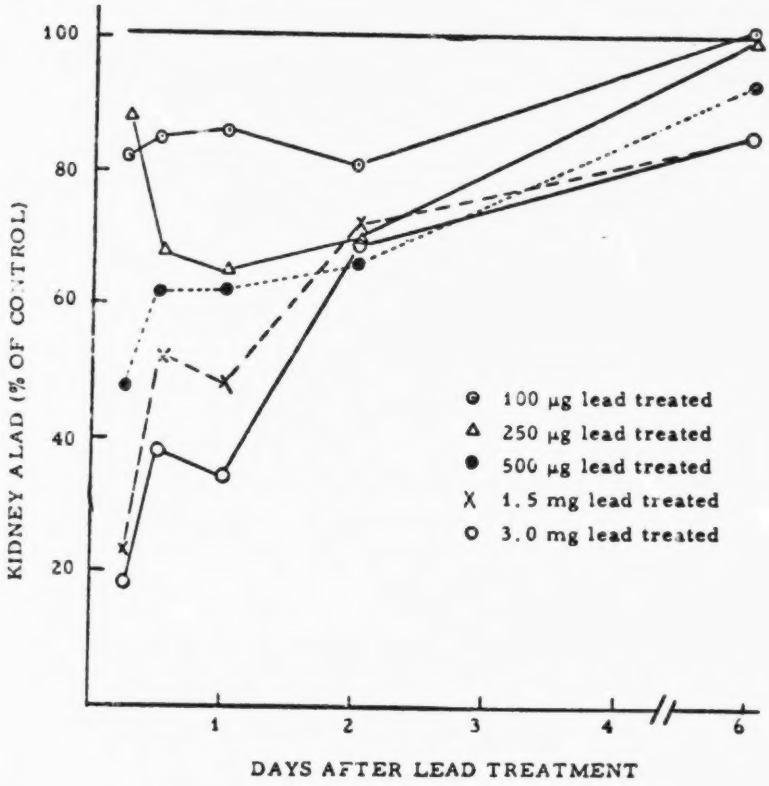


Figure 97. Effect of Lead on Kidney ALAD Activity.

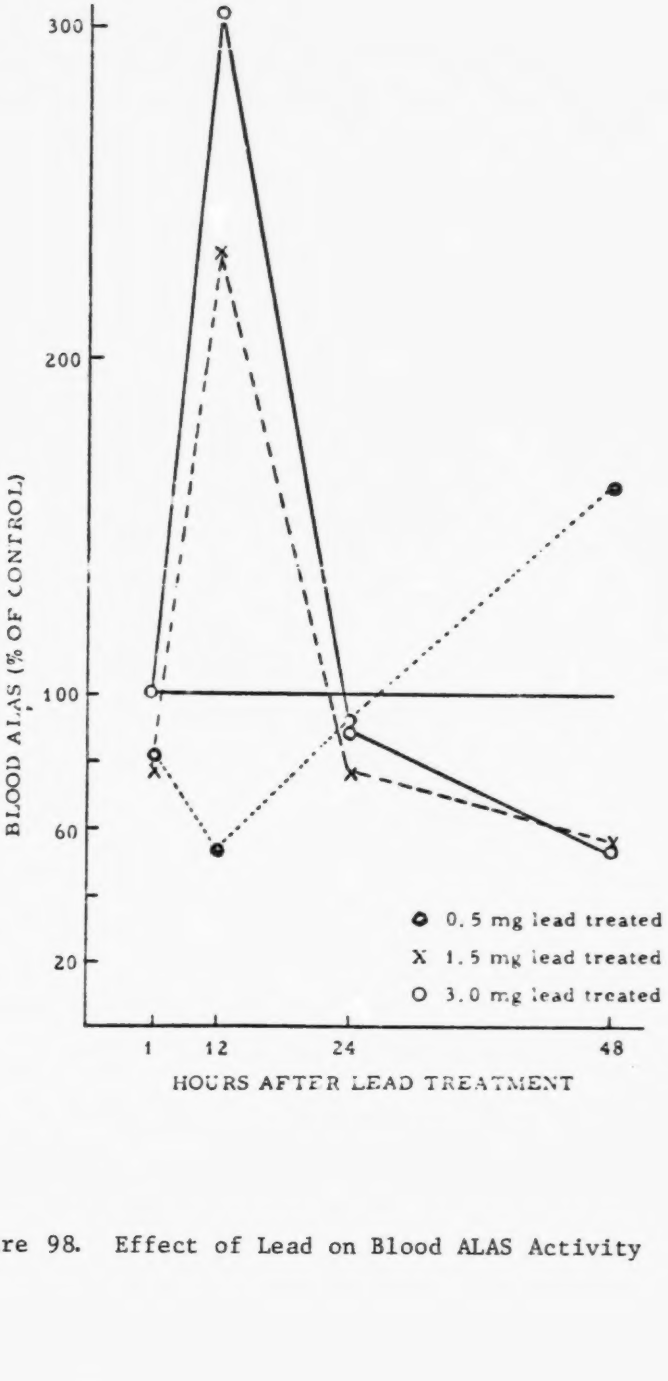


Figure 98. Effect of Lead on Blood ALAS Activity

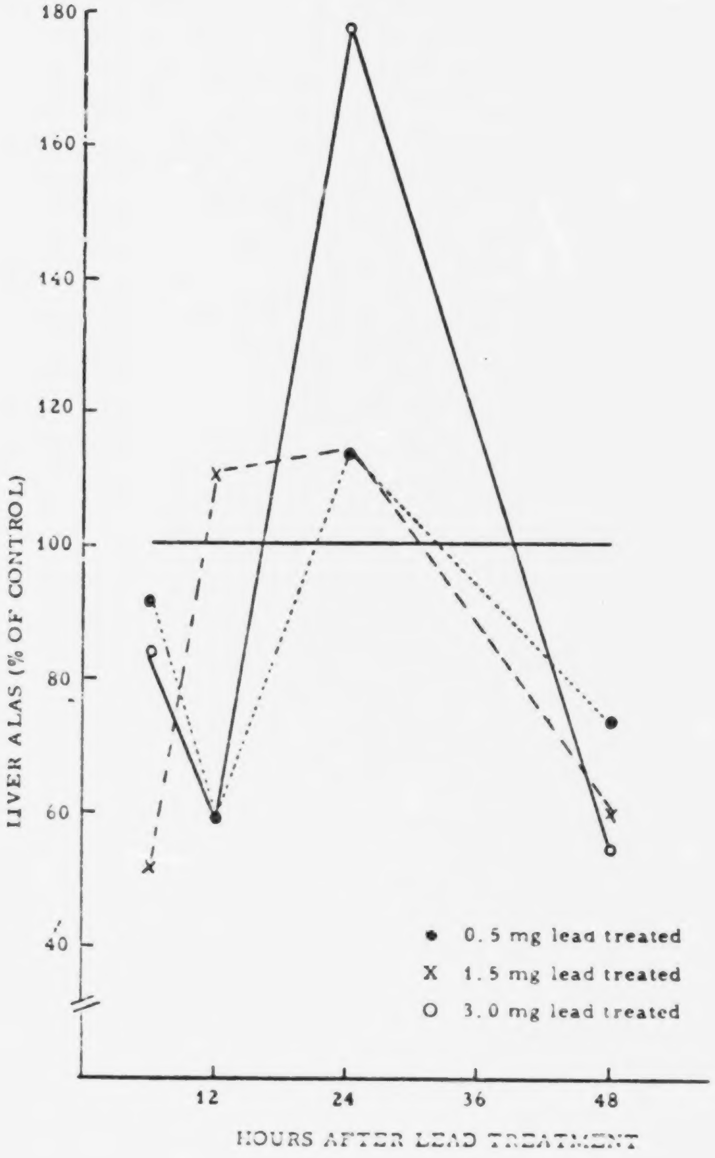


Figure 99. Effect of Lead on Liver ALAS Activity.

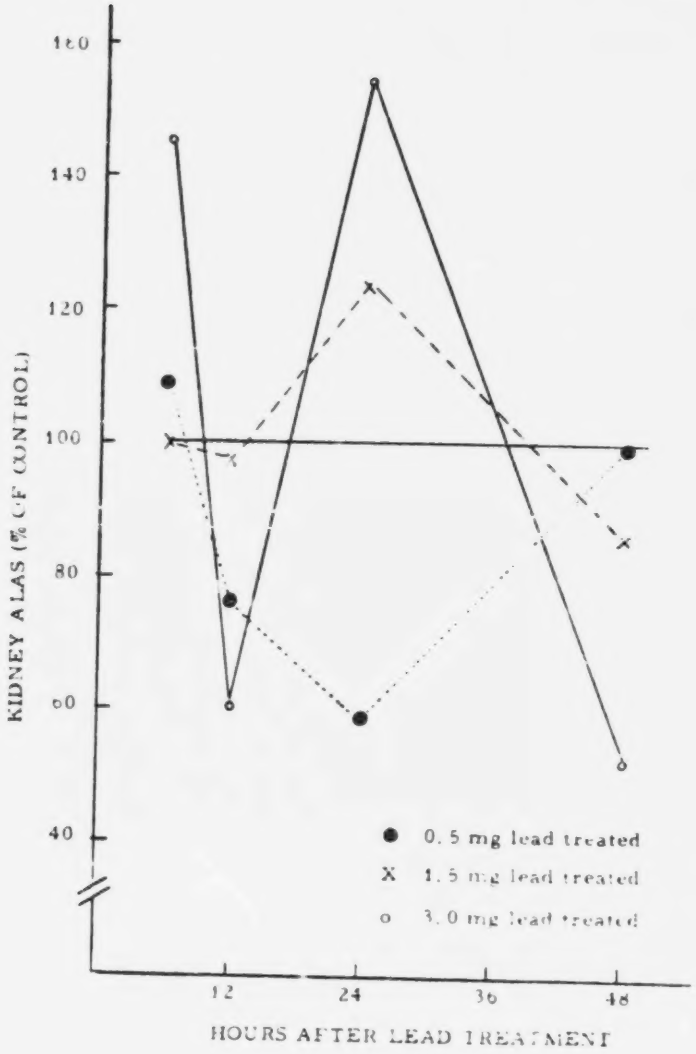


Figure 100. Effect of Lead on Kidney ALAS Activity.

Although there is quite a bit of variation here, especially in the ALAS data, taken as a whole it appears that ALA increases in urine as a result of both increased production and decreased utilization.

These data, expressed as % of control, do not tell the whole story. In looking at the raw data it was evident that only in blood does ALAS activity exceed that of ALAD in terms of ALA formed and utilized. Thus, if activities as measured in vitro are similar to the in vivo situation, only blood can serve as source of urinary ALA. This is illustrated in Table 41. The data do show that only when ALAS in blood exceeds ALAD does ALA in increased amounts appear in the urine. The fact that calculated excesses of ALA only fractionally appear in urine may be a result of supplying maximum conditions for enzyme action in vitro and also of the fact that other tissues may utilize blood-produced ALA, since it does freely pass membranes and since these tissues have an excess of ALAD.

c. Effects of lead on tryptophan pyrrolase. In the chick study concerned with effect of lead on tryptophane pyrrolase, lead did not influence weight gain or blood heme. Blood ALAD was reduced to 20, 9 and 2% of control values by lead and liver ALAD to 90, 73 and 62%. Liver tryptophan pyrrolase was not affected. Thus, in spite of the severe restriction in ALAD activity, we did not detect a difference in blood heme level or in activity of this liver heme enzyme in this short experiment. It is possible that other factors than available heme are limiting in the pyrrolase activity.

By way of summary of this portion of the work, we have some evidence that niacin in particular, but possibly also vitamin C and/or

Table 41
ALA Formed and Utilized in Blood and Excreted in Urine
(µg/hr)

Time After Dose	Lead Dosage (µg)	ALAS Activity	ALAD Activity	Excess ALA Formed	Urinary ALA
6 - 12 hr	0	61	186	-	3
	500	37	51	-	5
	1500	119	40	79	12
	3000	185	20	158	11
24 - 48 hr	0	75	129	-	3
	500	159	72	87	6
	1500	109	48	61	10
	3000	124	35	89	8

cobalamin, have an ameliorating effect on the heme synthesis defect in lead toxicity in the rat. We also have evidence that the increased ALA excretion in the urine of lead toxic animals is a response both to increased ALAS activity and to decreased ALAD activity.

C. Accumulation and Release of Lead From Animal Tissues

1. Methods.

Two hundred and forty adult male albine rats were involved in this experiment, being fed for a total of 8 weeks levels of 0, 20, 40, 80, 160 or 320 ppm lead, with some rats on each level being killed at 0, 1, 2, 4 and 8 weeks with the remaining rats being fed a total of 8 additional weeks on the 0 lead basal, again with some being killed at similar time intervals. Tissues were obtained for lead analysis and balance studies conducted at four intervals. It is hoped that this will provide the modeling team with data to develop rate constants for accumulation and disappearance of lead in animal tissues. Tissues were prepared for analysis by wet ashing (dry ashing for bone) and were then taken to the Environmental Research Laboratory for determination of lead by either atomic absorption or anodic stripping voltammetry.

2. Results.

The data thus far collected are shown in Table 42. Since these represent a small fraction of the data to be collected it is early to attempt an interpretation.

Table 42

Lead Storage in Animal Tissue
ppm Pb in fresh tissue *

Dietary Lead (ppm)	LIVER 7 days	LIVER 14 days	LIVER 28 days	SPLEEN 7 days	SPLEEN 14 days	SPLEEN 28 days	KIDNEY 7 days	KIDNEY 14 days	KIDNEY 28 days
0	<.15	.16 ± .09	.35 ± .22	.46 ± .15	.71 ± .35	1.1 ± .27	< .4	< .5	2.0 ± .9
20	.27 ± .01	.23 ± .11	.55 ± .30	.62 ± .26	.80 ± .61	1.7 ± .63	1.5 ± .38	1.3 ± .49	3.0 ± 1.1
40	.36 ± .07	.30 ± .12	.44 ± .09	.76 ± .21	.58 ± .10	1.2 ± .56	3.2 ± .90	1.9 ± .70	3.6 ± .72
80	.53 ± .13	.55 ± .16	.70 ± .53	.56 ± .11	1.13 ± .22	1.7 ± .97	4.6 ± .84	3.1 ± .60	3.5 ± 1.2
160	.59 ± .07	.57 ± .15	.77 ± .23	.86 ± .05	1.48 ± 1.06	2.3 ± 1.2	5.7 ± .65	3.4 ± .98	5.2 ± 1.6
320	1.18 ± .20	.79 ± .09	1.20 ± .32	1.0 ± .11	2.10 ± .86	2.5 ± .6	7.6 ± 1.2	5.3 ± 1.4	6.1 ± 3.0

Dietary Lead (ppm)	BLOOD 7 days	BLOOD 14 days	BLOOD 28 days	MUSCLE 7 days	MUSCLE 14 days	MUSCLE 28 days	BONE 7 days	BONE 14 days	BONE 28 days
0	.22 ± .13	.18 ± .03	.20 ± .12	.18 ± .10	.25 ± .11	.29 ± .02	.94 ± .34	.63 ± .16	.56 ± .32
20	.39 ± .17	.25 ± .08	.36 ± .11	.20 ± .06	.12 ± .04	.30 ± .10	1.71 ± .53	.84 ± .42	1.37 ± .71
40	.22 ± .05	.27 ± .07	.29 ± .11	.18 ± .24	.12 ± .02	.34 ± .15	2.19 ± .70	1.53 ± .90	2.98 ± 1.53
80	.27 ± .06	.35 ± .07	.48 ± .19	.39 ± .20	.11 ± .02	.28 ± .15	2.34 ± .42	2.54 ± 1.1	1.30 ± 1.0
160	.36 ± .09	.40 ± .11	.65 ± .20	.16 ± .04	.12 ± .05	.24 ± .03	2.78 ± .84	6.41 ± 4.36	10.3 ± 17.5
320	.42 ± .03	.39 ± .06	.59 ± .13	.25 ± .07	.11 ± .03	.30 ± .07	3.14 ± .65	11.92 ± 3.55	14.4 ± 18.6

* mean ± S. D., 5 animals/group

D. Personnel

Membrane-Lead Interactions

Dr. D. F. S. Natusch
Dr. G. L. Jendrasiak

Studies Related to Heme Synthesis

Dr. R. M. Forbes
Dr. R. L. C. Kao

Accumulation and Release of Lead from Animal Tissues

Dr. R. M. Forbes
Dr. R. L. C. Kao

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VIII. MODELING

INTRODUCTION

An interdisciplinary study of an environmental problem such as heavy metal pollution requires a framework to work within and a research plan leading toward the fulfillment of the study goals. The modeling team functions as an important unifying group within the heavy metals project. The framework of this particular study is a watershed ecosystem which provides a focal point. The goals of the study are to understand and model the movements and effects of heavy metals in the environment. Modeling is concerned with all parts of the project including mass transport of heavy metals and plant and animal effects. One important function of the modeling team is to synthesize and model information from all aspects of the project in order to work toward understanding the movements and effects of heavy metals in the ecosystem. A second function is to provide some guidance to project investigators as to the types of research which must be done in order to model various parts of the system.

The modeling team has a central staff supplemented at times by project investigators with expertise in specific problem areas. One typical example of this is the addition of Dr. Forbes to the modeling team when working with animal uptake and retention models since he can provide additional information in this area.

Future work will involve refinement of all models and additional air transport work.

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A. Transport of Lead in the Environment

1. Objectives

The modeling group has as one specific objective the modeling of lead transport in the environment. That is, to predict where lead goes and how much is located in various environmental components, to evaluate the effects, to predict the future transports, and to suggest what effects might occur due to changing the major sources of lead in the environment. It was decided to model a small watershed as a prototype or pilot study for modeling large segments of the environment. The objectives are to test the model by correlating it with field data collected in the watershed and to suggest possible questionable areas in the lead transport problem which might be studied as part of the other research projects. The study of lead transport in the environment will help to define the input for other models being developed for animals and for plants.

2. The Watershed

The 76-square-mile watershed selected for the study lies north and east of Champaign-Urbana, Illinois, in Champaign County. The U.S. Geological Survey stream gage which lies north of Mayview, Illinois, five miles east of Urbana, will serve as the main outlet. Boneyard Creek, a totally urban drainage channel, joins the primary channel, Saline Branch Drainage Ditch, on the northeast edge of Urbana.

Lead input to the watershed will be determined by traffic volume data converted to gallons of gasoline consumed within the watershed. The conversion of gasoline consumption to lead emitted will then be estimated. Lead output and movement within the watershed will be determined by monitoring and sampling stream water at five locations within the watershed. Soils, plants, and animals will also be sampled to determine lead concentrations in these components.

The Saline Branch Watershed-Ecosystem has many advantages for this type of study. Being immediately adjacent to Champaign-Urbana, it provides easy access, and a wealth of background information is presently available. The watershed contains five distinct compartments of land use which can be examined separately or as a unit. These compartments are: 1) cultivated cropland, 2) pasture or sod cropland, 3) forest, 4) residential and urban development, and 5) wasteland (railroad right-of-ways, roadsides, fencerows, etc.). The watershed also has a typical urban/rural balance for the Midwest.

3. The Flow Model

A model was developed to study the flow of lead or other heavy metals through an ecosystem. The main concepts involved will be reviewed in this section.

The ecosystem is represented by a network where the nodes stand for the various compartments of the ecosystem and the branches indicate possible transport mechanisms between nodes.

Two attributes are associated with each node: 1) a mass (to compute concentrations) and 2) a quantity of lead input per time period for source nodes.

Distribution factors and seasonal factors are used to quantitatively describe the flows in the branches. The distribution factors specify the percentage of the lead content of a node which flows in branches emanating from that node. These distribution factors can be random variables following various probability distributions (see Figure 101.) It is recognized that the flows are affected by the seasons of the year. This is accounted

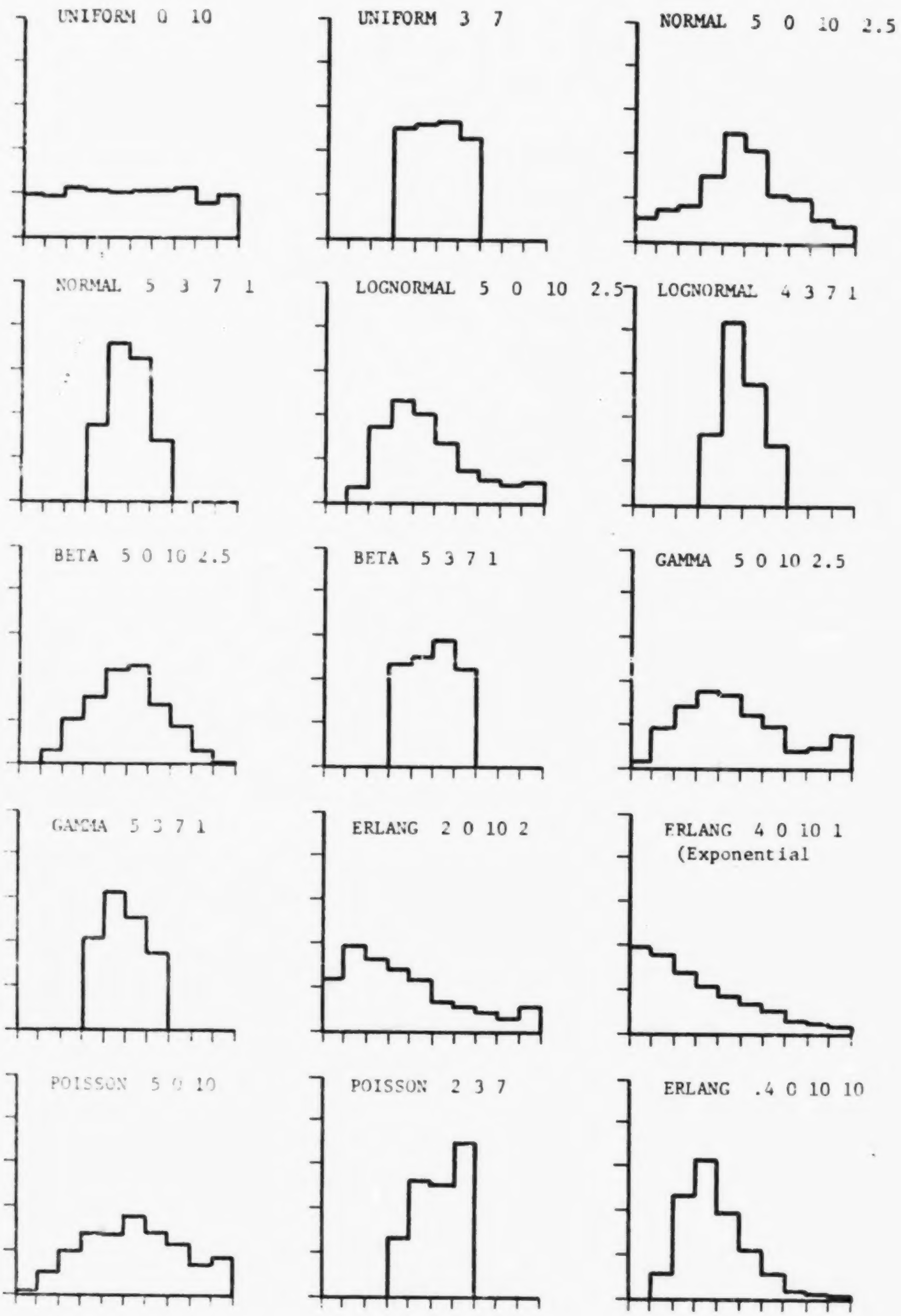


Figure 101.Example of 1000 Sample Distribution Factors.

for by modifying the distribution factor of each branch according to the seasonal factor.

Simulation proceeds in the following manner. For each cycle (time period) and for each branch in the network, the program computes a distribution factor and adjusts it according to the seasonal factor. Then for each node, the sum of the distribution factors for those branches exiting the node is computed. The branch flows are then sent to the ending node of each branch in proportion to the distribution factor for that branch divided by the sum of the distribution factors of all the branches leaving from that node. Equilibrium (i.e., mass balance) is thus maintained.

The computer program which was developed to simulate the flow of a substance through a network uses a problem-oriented language (POL) whereby the ecosystem model can be described in free form.

The network representing the ecosystem is described in terms of nodes, branches, and branch properties (distribution factors, seasonal factors). Some information is needed to set up the simulation conditions (cycles to be traced, length of the simulation period in number of cycles). The desired output can also be specified.

The program provides the user with a printout of the input data, along with statistics of nodal accumulations (average, standard deviation, minimum, maximum) and concentrations (average and maximum). Plots of nodal accumulations or concentrations versus time can be obtained.

4. Simple Watershed Model

The basic system model is described in Figure 102. The nodes, shown as boxes, represent various states in which lead or other heavy metals or pollutants might exist. For example, node 50 represents all of the lead in

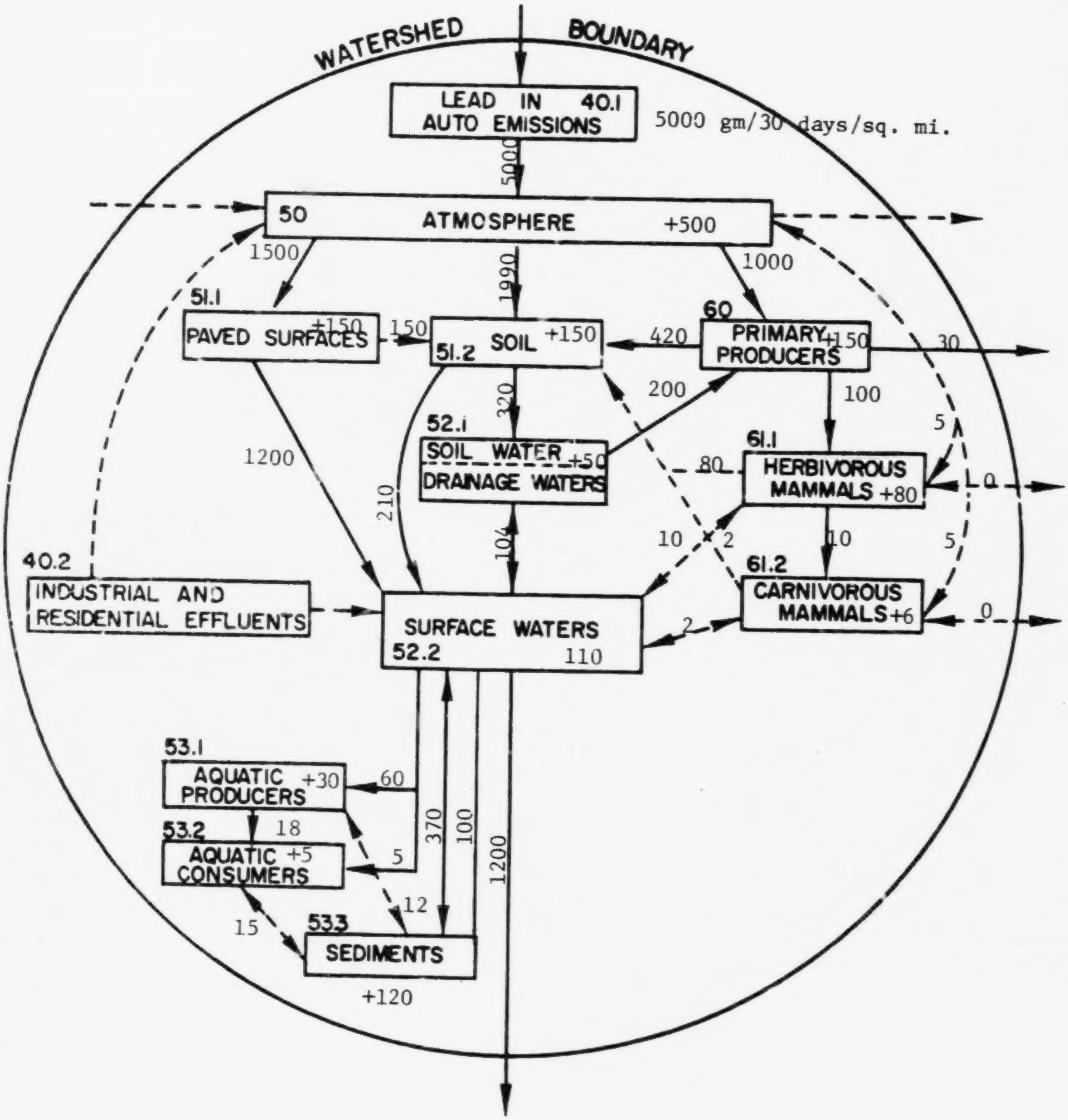

WATERSHED BOUNDARY
LEAD IN 40.1
AUTO EMISSIONS
5000 gm/30 days/sq. mi.
5000
50
ATMOSPHERE
+500
1500
1990
1000
51.1
PAVED SURFACES
+150
150
51.2
SOIL
+150
420
60
PRIMARY PRODUCERS
+150
30
320
200
100
52.1
SOIL WATER
DRAINAGE WATERS
+50
1200
80
61.1
HERBIVOROUS MAMMALS
+80
5
0
210
104
10
2
10
5
40.2
INDUSTRIAL AND RESIDENTIAL EFFLUENTS
61.2
CARNIVOROUS MAMMALS
+6
0
2
SURFACE WATERS
52.2
110
53.1
AQUATIC PRODUCERS
+30
60
18
53.2
AQUATIC CONSUMERS
+5
5
370
100
1200
12
15
53.3
SEDIMENTS
+120

Figure 102. Basic System Model.

the atmosphere over the watershed at a particular time. The arrows represent possible transport to other nodes, e.g., the atmosphere transports lead to the soil, to water, to primary producers, and to paved surfaces. The atmosphere may receive lead from the industrial and residential areas and from auto emissions both inside and outside the watershed boundary. Also, lead may be transported to and from this particular watershed and adjacent watersheds.

The branches with two arrowheads indicate that there is a possible transport of lead in both directions. The transport may vary as a function of the season of the year and as a function of other factors such as environmental conditions and the concentration of lead in a particular node at a particular time. Feedback-loops (not shown) represent that portion of the pollutant, in this case lead, which remains in a particular node from one time period to another.

A network representation of this model appears in Figure 103. The node representing industrial and residential effluents has been omitted, and will initially be considered negligible. Two sink nodes have been added (soil sink and sediment sink). The soil sink, for example, represents a portion of the soil which holds lead and does not release it to other environmental compartments.

The modeling group developed the model by consulting with various project investigators. Estimates of distribution patterns and compartment masses have been obtained from these investigators. A refined estimate of lead input from auto emissions (12,000 g/week/square mile) was obtained using data on gasoline consumption, vehicle-miles traveled, and population statistics.

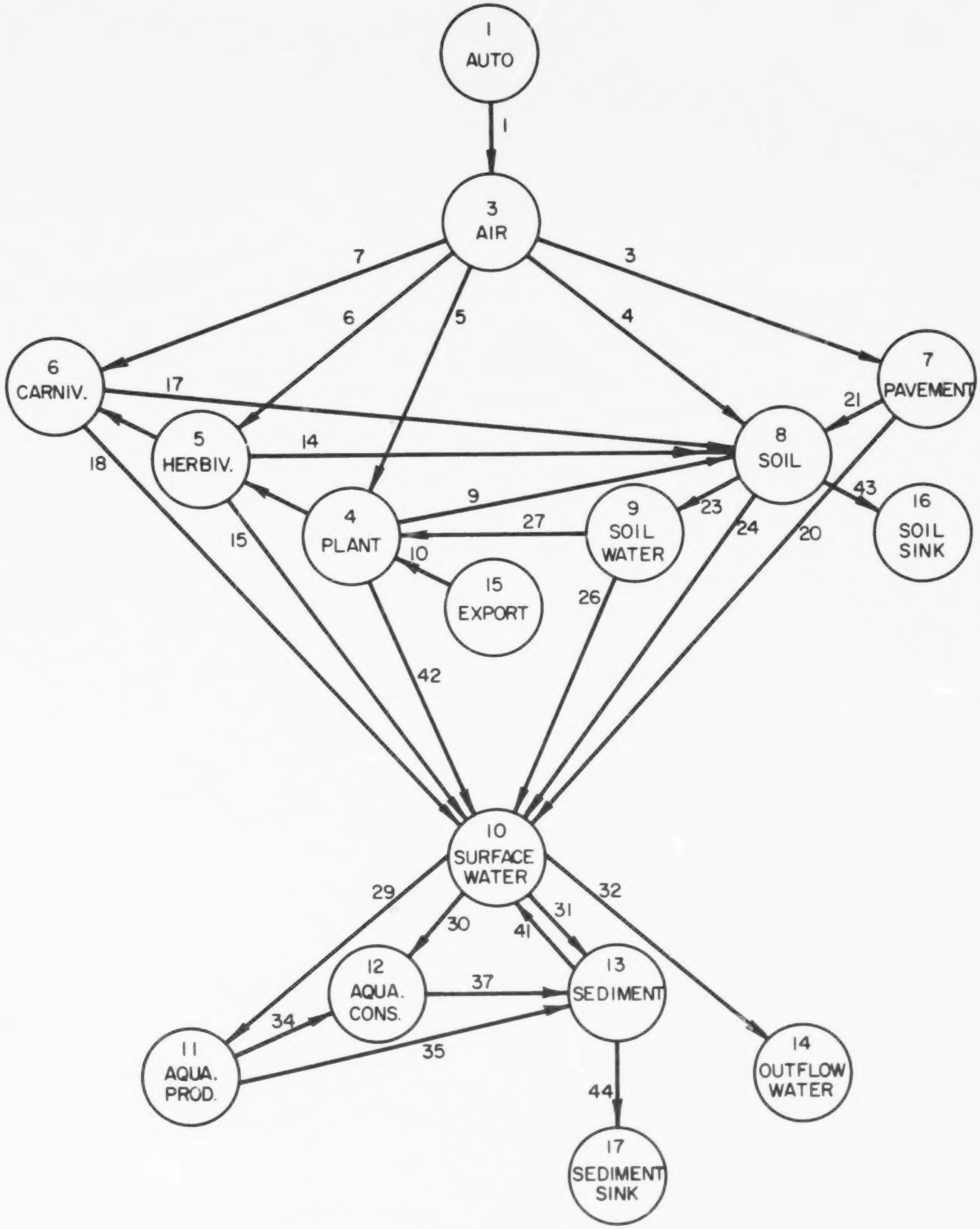

1
AUTO
1
3
AIR
7
6
5
4
3
6
CARNIV.
17
7
PAVEMENT
21
5
HERBIV.
14
8
SOIL
18
9
4
PLANT
27
9
SOIL
WATER
23
43
16
SOIL
SINK
15
10
24
20
15
EXPORT
26
42
10
SURFACE
WATER
29
32
30
31
41
12
AQUA.
CONS.
37
13
SEDIMENT
34
35
11
AQUA.
PROD.
44
14
OUTFLOW
WATER
17
SEDIMENT
SINK

Figure 103. Basic Network Model

The input data describing this model to the computer appear in Figures 104 and 105. The model has 17 nodes and 44 branches. Each node is described by specifying the node number, a title, and the estimated mass of that particular node, followed by the quantity of lead input per cycle. In this case, we have put in 12,000 grams of lead from auto emissions every cycle (week). Each branch is then described by giving the branch number, the node where it starts, the node where it ends, and the distribution factor for that particular branch. A seasonal factor is also specified for each branch in order to account for the seasonal variation of the distribution factor. The seasonal factors are shown in Figure 106. For example, the seasonal factor for the transport from air to plants (number 2) indicates that when the plants are not available to any considerable amount (during January and February), the distribution of lead to the plants will be very small. During July and August, the distribution to the plants will be at a maximum. Then it will drop off in October and gradually decline until January and February.

Typical results of a 100-cycle simulation (approximately two years) with no initial lead concentrations are shown in Figures 107, 108, 109, and 110. Figure 110 shows those sinks which have been set up in the system. The soil sink and water outflow receive approximately 50 percent of the lead input to the system.

5. Expanded Watershed Model

The simple model presented in the previous section was expanded because it implicitly assumes that the watershed is homogeneous. The non-uniform nature of the watershed was taken into account by breaking it down into two areas according to the estimated input of lead: 1) a rural area which consists

```
 NUMBER OF NODES 17 BRANCHES 44
NODES
1 "40.1 AUTO EMISSIONS " 1.58E-3 12000.
2 "40.2 INDUST + RESIDENTIAL EFFLUENTS" 1.58E-3
3 "50. ATMOSPHERE" 5.44E3
4 "60. PLANTS + LITTER" 9.2E2
5 "61.1 HERBIVOROUS MAMMALS (DOM+WILD)" 5.13
6 "61.2 CARNIVOROUS MAMMALS" 1.27
7 "51.1 PAVED SURFACE" 4.06E3
8 "51.2 SOIL" 406.E3
9 "52.1 DRAINAGE WATER" 12.05E2
10 "52.2 SURFACE WATER" 12.05E3
11 "53.1 AQUATIC PRODUCERS" 1.32E2
12 "53.2 AQUATIC CONSUMERS" 1.32E1
13 "53.3 SEDIMENTS" 1.205E4
14 "100. EXIT WATER OUTFLOW" 1.205E6
15 "100.6 EXIT PLANT EXPORT " 5.44E4
 16 "SOIL SINK" 406.E3
 17 "SEDIMENT SINK" 1.205E5
BRANCHES
1 1 3 CONSTANT 100
2 3 3 CONSTANT 1000
3 3 7 BETA 5000 3000 7000 100000
4 3 8 BETA 2480 1480 3480 80000
5 3 4 BETA 1500 1200 1800 20000
 6 3 5 BETA 10 1 20 1
 7 3 6 BETA 10 1 20 2
 8 4 4 CONSTANT 22
 9 4 8 BETA 45 37 53 10
10 4 15 BETA 15 10 20 2
11 4 5 BETA 8 6 10 1
 12 5 5 BETA 50 40 60 3
 13 5 6 BETA 100 60 140 300
 14 5 8 BETA 750 600 900 4000
 15 5 10 BETA 100 60 140 300
16 6 6 CONSTANT 5
 17 6 8 BETA 80 64 96 4
 18 6 10 BETA 15 12 18 .5
 19 7 7 CONSTANT 10
 20 7 10 ERLANG 80 5 96 1
 21 7 8 ERLANG 10 .5 12 1
 22 8 8 CONSTANT 8
 23 8 9 ERLANG 12 6 16 1
 24 8 10 ERLANG 8 4 12 1
 25 9 9 BETA 5 4 6 .2
 26 9 10 BETA 33 27 40 1.5
 27 9 4 BETA 62 50 74 3
 28 10 10 BETA 65 52 75 3
 29 10 11 BETA 34 27 41 1
 30 10 12 BETA 3 2 4 .1
 31 10 13 BETA 210 170 250 1100
 32 10 14 BETA 680 540 820 3400
 33 11 11 BETA 50 40 60 2.5
 34 11 12 BETA 30 24 36 1.5
 35 11 13 BETA 20 16 24 1.
 36 12 12 BETA 33 26 40 2.
 37 12 13 BETA 67 54 77 4.
 38 13 13 CONSTANT 37.5
 39 14 14 CONSTANT 100
```

Figure 104. Basic Network Input Data

```
40 15 15 CONSTANT 100
41 13 10 BETA 25 20 30 1.2
42 4 10 ERLANG 10 1 30 1.0
43 8 16 CONSTANT 72
44 13 17 CONSTANT 37.5
NUMBER OF TERMS 12 SPACE 4
FACTORS
1 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1.
2 .48 .48 .64 .96 1.44 1.6 1.6 1.6 1.28 .8 .64 .48
3 .0 .0 .25 .75 2. 2.5 2.5 2.5 1.25 .25 .0 .0
4 .8 .8 .8 .4 .4 .6 .6 .8 1.4 2.0 1.4 .8
5 1.43 1.43 1.29 1.0 .57 .43 .43 .43 .86 1.14 1.29 1.43
POINTERS
1 1 2 1 3 1 6 1 7 1 8 1 11 1 12 1 13 1 14 1 15 1
15 1 16 1 17 1 18 1 19 1 20 1 21 1 22 1 23 1 24 1
25 1 26 1 28 1 31 1 32 1 33 1 36 1 37 1 38 1 39 1 40 1
41 1 43 1 44 1
4 5 5 2 9 6 10 4 11 2 27 3 29 2 30 2 34 2 35 4 42 2
TRACE 4 100 4 CONCENTRATIONS
NUMBER OF CYCLES 100 SOLVE OUTPUT
```

Figure 105. Basic Network Input Data
(continued)

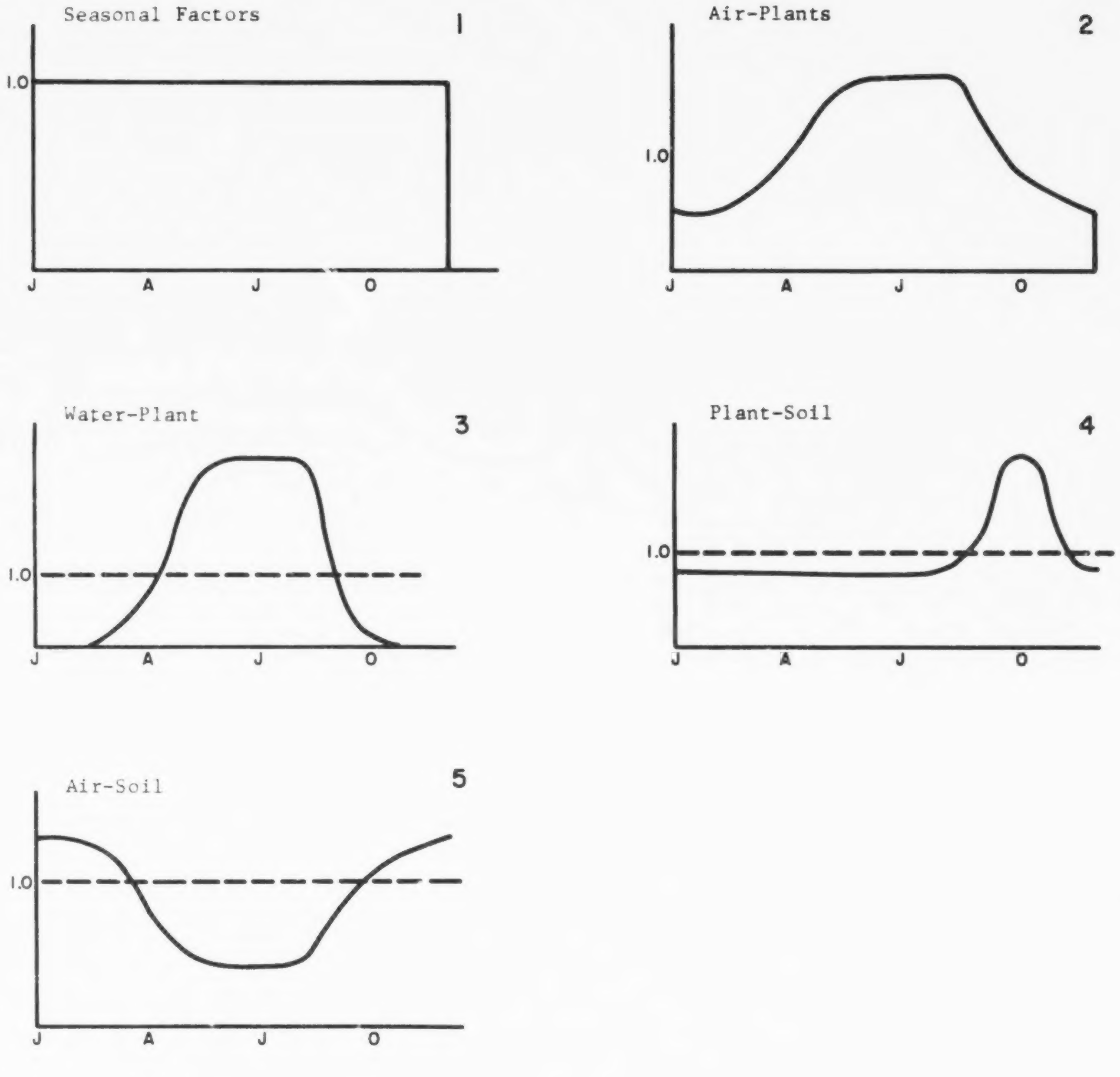


Figure 106. Seasonal Factors

```
NODE  DESCRIPTION                               MIN          AVE          MAX          DEV      AVE CONC    MAX CONC

  1 40.1 AUTO EMISSIONS                        0.000        0.000        0.000        0.000    0.00000     0.00000
  2 40.2 INDUST + RESIDENTIAL FUEL             0.000        0.000        0.000        0.000    0.00000     0.00000
  3 50. ATMOSPHERE                          1167.437     1337.496     1570.573       84.645    0.24586     0.28971
  4 60. PLANTS + LITTER                      240.444      903.214     2301.645      549.386    0.98252     2.50179
  5 61.1 HERBIVOROUS MAMMALS (COW+MI           2.560       16.188       49.605       14.015    3.15564     9.68722
  6 61.2 CARNIVOROUS MAMMALS                   0.767        2.380        6.527        1.509    1.97412     5.21827
  7 51.1 PAVED SURFACE                       605.005     1744.276     5630.303     1007.406    0.42962     1.38677
  8 51.2 SOIL                                351.568      532.304      769.925       96.646    0.00131     0.00190
  9 52.1 DRAINAGE WATER                        8.401       58.280      100.187       44.057    0.04836     0.16530
 10 52.2 SURFACE WATER                       700.053     1336.155     1006.315      239.673    0.11088     0.16567
 11 53.1 AQUATIC PRODUCERS                    71.319      265.750      480.904       79.721    2.01326     3.67420
 12 53.2 AQUATIC CONSUMERS                    11.004       96.602      236.226       65.200    7.31832    17.80595
 13 53.3 SEDIMENTS                           502.328     1208.685     1747.234      218.901    0.10031     0.14666
 14 100. EXIT WATER OUTFLOW                 3857.806   270437.748   551121.181   161309.880    0.23190     0.45736
 15 100.5 EXIT PLANT EXPORT                  130.540    20341.439    40853.265    12690.055    0.37392     0.75098
 16 SOIL SINK                               3403.022   237881.224   479073.649   136487.545    0.58591     1.17908
 17 SEDIMENT SINK                            502.328    60837.568   120868.537    36065.206    0.50488     1.00306
 PLOT 3 4 7 8
NOK
```

Figure 107. Basic Network Output: Statistical Results

```
NODE LABEL                                   KEY
   3 50. ATMOSPHERE                           3
   4 60. PLANTS + LITTER                      4
   7 51.1 PAVED SURFACE                       7
   8 51.2 SOIL                                8
```

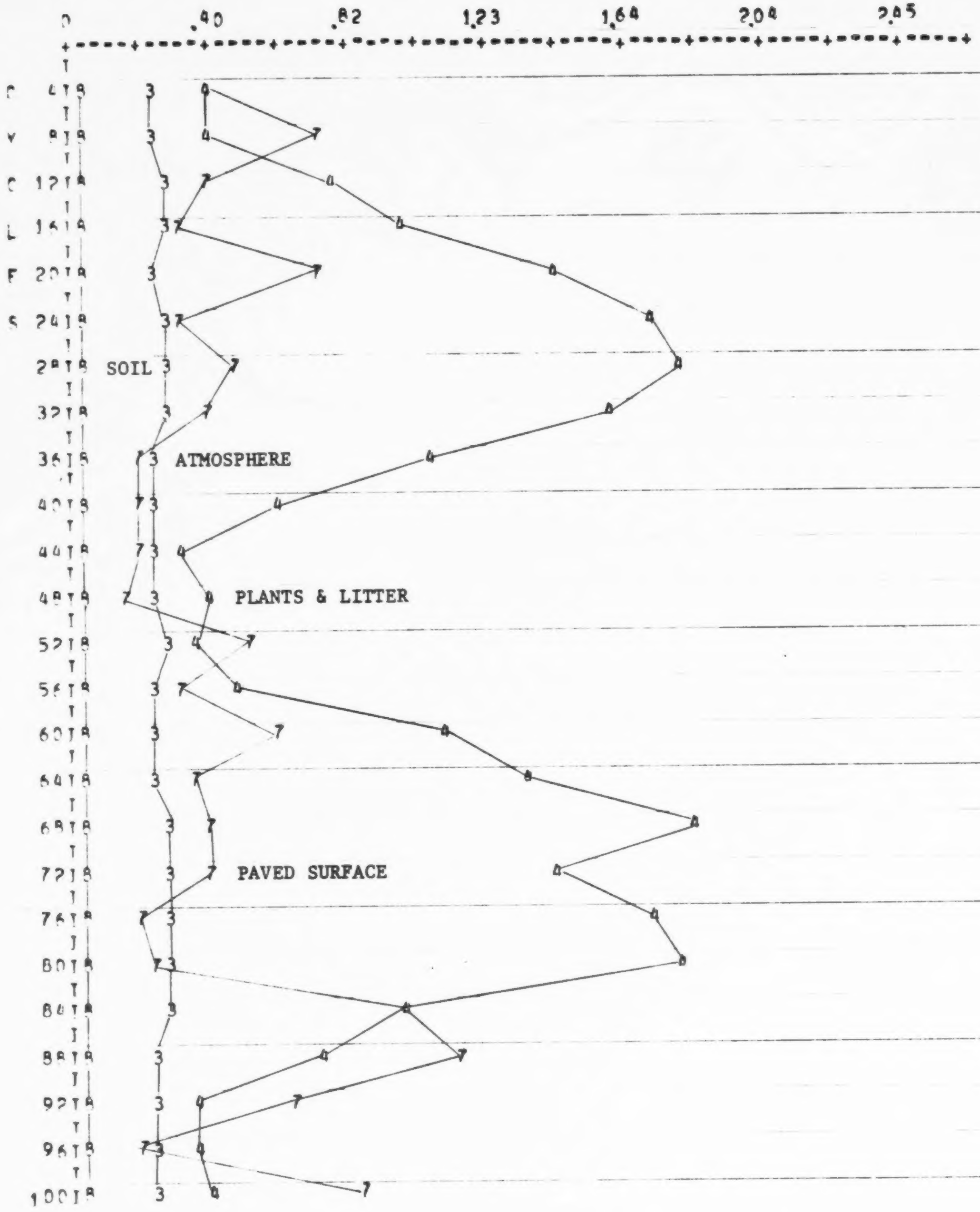


Figure 108. Basic Network Output: Plot of Atmosphere, Plants and Litter, Paved Surface, Soil

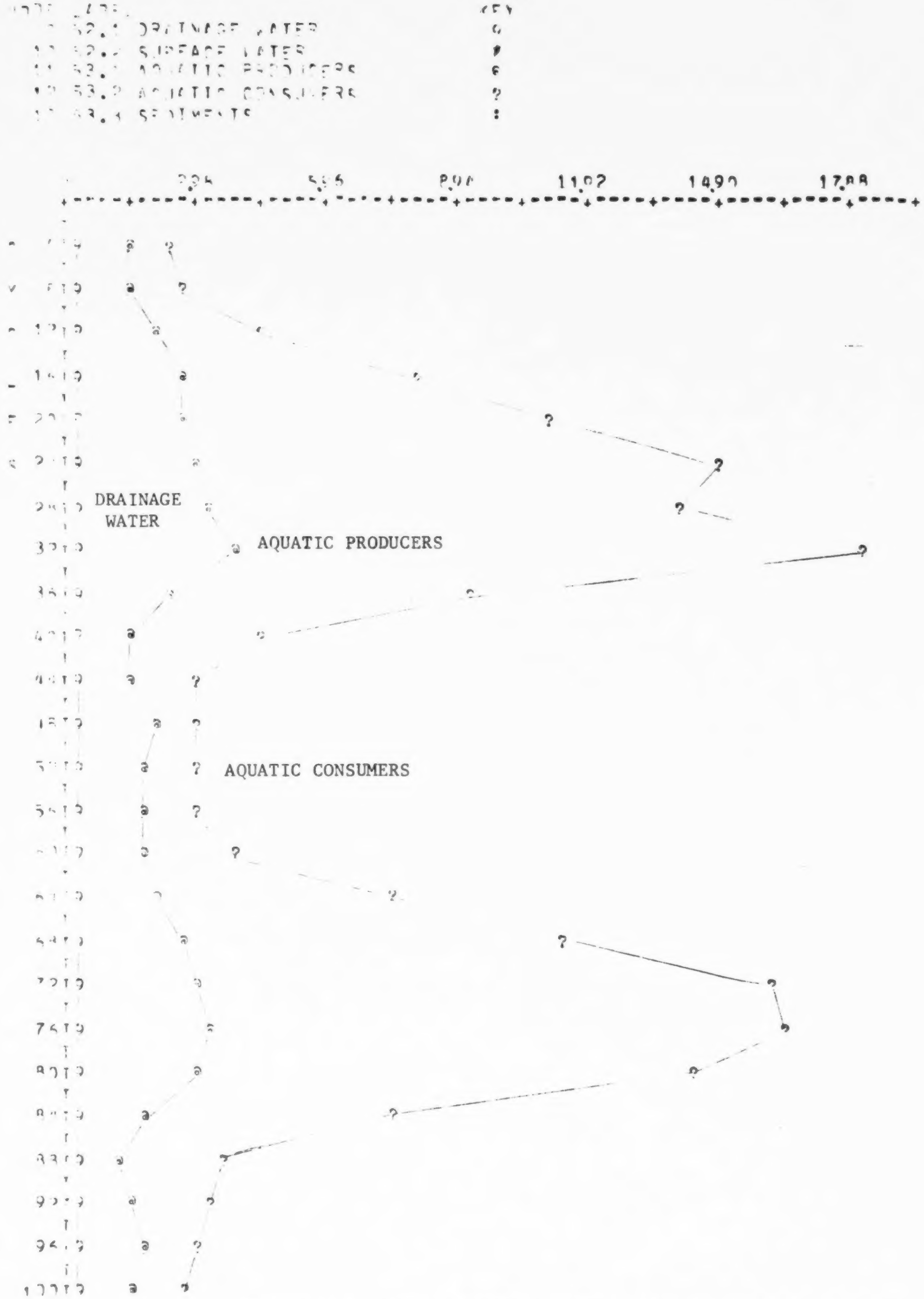


Figure 109. Basic Network Output: Plot of Drainage Water, Aquatic Producers and Consumers

```
NODE LABEL                              KEY
 14 100. EXIT WATER OUTFLOW              >
 15 100.6 EXIT PLANT EXPORT              >
 16 SOIL SINK                            +
 17 SEDIMENT SINK                        A
```

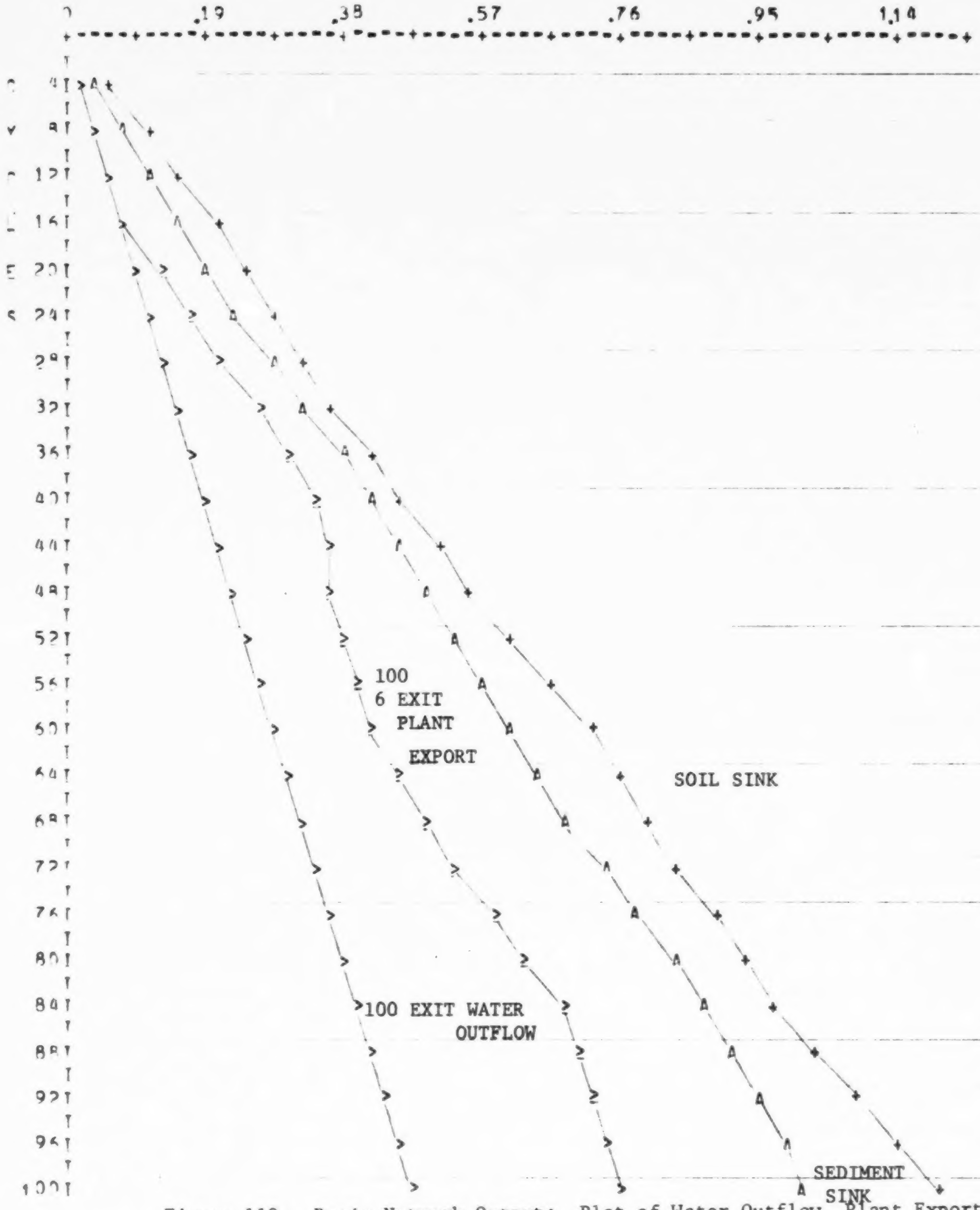


Figure 110. Basic Network Output: Plot of Water Outflow, Plant Export, Sediment and Soil Sinks

of three zones, and 2) an urban area. These areas are shown by Map 1 . Further divisions will ultimately be necessary.

The rural area will be divided into three zones based on the amount of lead deposited in the soil (as roughly determined by traffic volume). Zones 1 and 2 will essentially consist of strips of ground along the roads within the watershed. For north-south roads, this will be a strip 30 m wide on the west side of the road and 50 m wide on the east side to account for the prevailing winds; while for east-west roads, it will be a 40 m strip on each side. Zone 1 will be a high-input zone along heavily traveled highways with 4000 or more vehicles/24 hours, and Zone 2 will be the intermediate-input zone with less than 4000 vehicles/24 hours. Zone 3 will be a low-input area consisting of all land within the rural portion of the watershed not bordering on a roadway. The urban area will not be divided into zones initially.

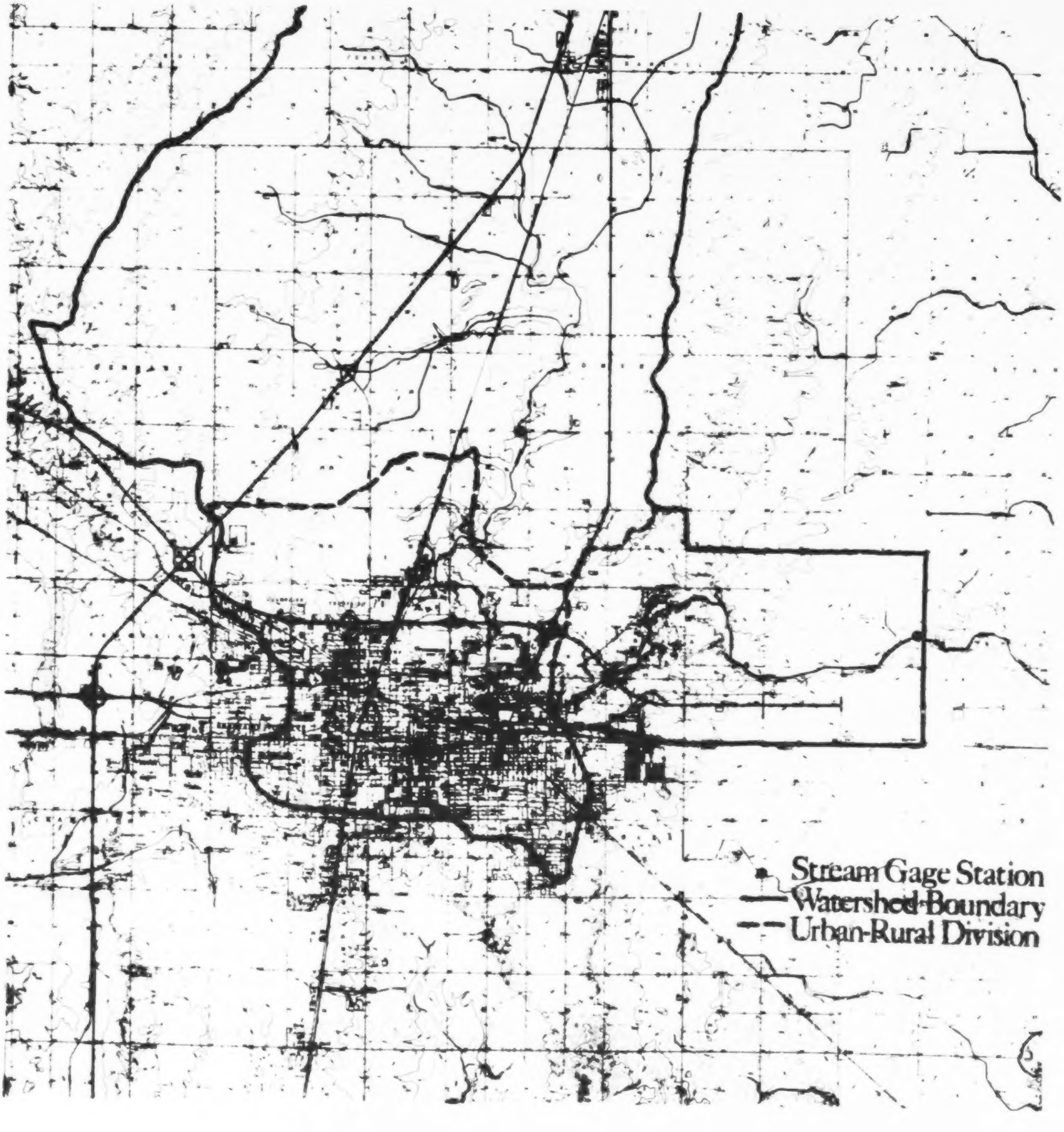
The percentage of the total lead input going into each zone and the percentage of the total area occupied by each zone was estimated by project investigators and is shown in Table 43.

This model expansion resulted in network changes. Some of the nodes were replicated four times, one for each zone: plants, herbivores, carnivores, soil, soil sink, and drainage water. Three pavement nodes were used (no pavement in Zone 3). The necessary branches were added. The new network, which consists of 36 nodes and 121 branches, is shown in Figure 111.

Refined estimates of compartment biomasses, including a breakdown by zone, were obtained from the investigators. Input data for this model are a refinement of that shown in Figures 104 and 105. The results of a two-year simulation (Figure 112) show for each compartment:

the minimum quantity of lead

the maximum quantity of lead


Stream Gage Station
Watershed Boundary
Urban-Rural Division

Map 1. Saline Branch Watershed.

Table 43.

Lead Input and Area by Zones

	Rural			Urban
Zone	1	2	3	4
Percent Area	8.5	17	59.5	15
Percent Lead Input	15	4	1	80

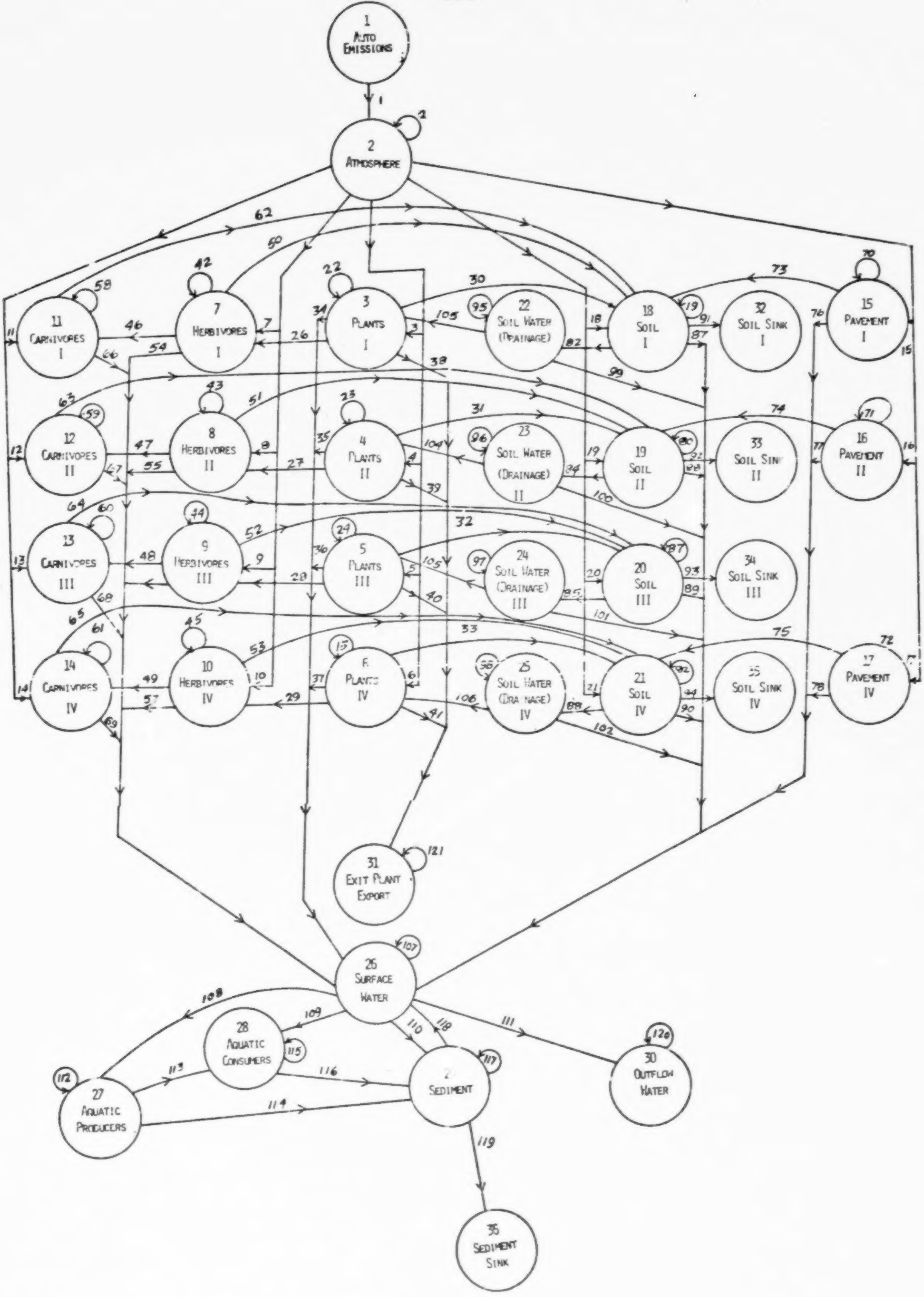

1 Auto Emissions
2 Atmosphere
3 Plants I
4 Plants II
5 Plants III
6 Plants IV
7 Herbivores I
8 Herbivores II
9 Herbivores III
10 Herbivores IV
11 Carnivores I
12 Carnivores II
13 Carnivores III
14 Carnivores IV
15 Pavement I
16 Pavement II
17 Pavement IV
18 Soil I
19 Soil II
20 Soil III
21 Soil IV
22 Soil Water (Drainage) I
23 Soil Water (Drainage) II
24 Soil Water (Drainage) III
25 Soil Water (Drainage) IV
26 Surface Water
27 Aquatic Producers
28 Aquatic Consumers
Sediment
30 Outflow Water
31 Exit Plant Export
32 Soil Sink I
33 Soil Sink II
34 Soil Sink III
35 Soil Sink IV
35 Sediment Sink

Figure 111. Expanded Network Model

```
NODE  DESCRIPTION                           MIN          AVE          MAX          DEV      AVE CONC    MAX CONC

  1 AUTO EMISSIONS                         0.000        0.000        0.000        0.000     0.00000     0.00000
  2 ATMOSPHERE                          1154.217     1344.310     1528.464       70.165     0.24712     0.28097
  3 PLANTS + LITTER          I            35.248      132.185      284.738       76.443     0.08011     0.17257
  4 PLANTS + LITTER          II            9.055       37.754       94.948       23.919     0.01144     0.02977
  5 PLANTS + LITTER          III           2.468        8.356       12.397        4.921     0.00042     0.00097
  6 PLANTS + LITTER          IV          161.678      740.352     1603.605      457.893     0.63278     1.45180
  7 HERBIVORS                I             0.406        2.281        8.235        1.924     5.23253    18.88691
  8 HERBIVORS                II            0.103        0.653        2.445        0.573     0.74694     2.79799
  9 HERBIVORS                III           0.031        0.151        0.420        0.128     0.04947     0.14746
 10 HERBIVORS                IV            1.722       12.484       34.937       10.491    16.21354    45.37279
 11 CARNIVORS                I             0.140        0.330        0.936        0.197     3.13660     8.67073
 12 CARNIVORS                II            0.027        0.094        0.245        0.059     0.43481     1.13499
 13 CARNIVORS                III           0.007        0.023        0.072        0.014     0.03016     0.09543
 14 CARNIVORS                IV            0.452        1.942        4.818        1.170    10.22236    25.35705
 15 PAVEMENT                 I            90.681      237.961      738.767      150.851     0.00071     0.00220
 16 PAVEMENT                 II           28.425       76.688      315.180       56.403     0.00008     0.00031
 17 PAVEMENT                 IV          429.491     1275.010     4070.569      840.748     0.00033     0.00106
 18 SOIL                     I            46.390       79.363      135.510       18.378     0.04587     0.07833
 19 SOIL                     II           14.568       21.896       30.075        4.488     0.00633     0.00985
 20 SOIL                     III           2.761        4.331        6.456        0.818     0.00036     0.00054
 21 SOIL                     IV          260.735      422.972      505.717       71.203     0.46226     0.65106
 22 SOIL WATER (DRAINAGE)    I             1.001        8.642       28.440        6.727     0.08473     0.27883
 23 SOIL WATER (DRAINAGE)    II            0.320        2.431        8.577        1.831     0.01186     0.04184
 24 SOIL WATER (DRAINAGE)    III           0.057        0.492        1.462        0.382     0.00069     0.00204
 25 SOIL WATER (DRAINAGE)    IV            6.110       44.528      173.842       33.105     0.24601     0.96045
 26 SURFACE WATER                        779.069     1325.093     1783.944      221.364     0.10997     0.14805
 27 AQUATIC PRODUCERS                     61.543      264.200      416.142       78.507     2.00154     3.15259
 28 AQUATIC CONSUMERS                      9.063       37.990      230.524       67.048     7.41660    17.46620
 29 SEDIMENT                             520.951     1189.714     1677.290      192.433     0.09873     0.13919
 30 OUTFLOW WATER                       3775.393   282124.332   559037.208   161979.794     0.23413     0.46310
 31 EXIT PLANT EXPORT                    122.075    [illegible]351.286    40906.155    12563.458     0.37410     0.75177
 32 SOIL SINK                I           664.[illegible]    [illegible]934.850    71026.594    20233.902     1.09224     2.17102
 33 SOIL SINK                II          181.0[illegible]     9819.213    19706.735     5656.105     0.14946     0.29995
 34 SOIL SINK                III          35.803     1032.702     3807.639     1106.209     0.00840     0.01695
 35 SOIL SINK                IV         2854.636   189202.285   380670.376   110165.015    10.87369    21.87785
 36 SEDIMENT SINK                        520.951    59303.184   118371.440    34887.719     0.49214     0.98731
PLOT 7 11 32 NOW
```

Figure 112. Expanded Network Output: Statistical Results.

the average quantity of lead

the standard deviation of the quantity of lead

the average concentration

the maximum concentration.

Table 44 shows the average lead concentration (in ppm) in a few key compartments.

Typical plots of concentration versus time are shown in Figures 113, 114, 115, and 116 for some nodes in Zones I and II. The cyclic behavior due to seasonal variations can be easily observed. The concentration in some sinks (outflow, exit plant export, soil sinks) keeps increasing (Figures 117 and 118.)

6. Comparisons

The breakdown by zones produces more meaningful results. For example, concentrations in plants are given for each zone and it is easier to compare the results of the simulation with field data. The same comment applies to herbivores, carnivores, soil, and water. It can also be observed that the sum of the lead content of the 4 plant nodes, for example, is equal to the lead content of the plant node of the simple watershed model.

7. Conclusions and Further Study

The flow model can be used to study the transport of lead in the environment, as shown by the two examples. The reliability of the entire model, however, is dependent upon the determination of the branch distribution factors which again are dependent upon the knowledge of the lead transport between compartments. Field and laboratory experiments are continually being conducted to further our knowledge of these transports. After

Table 44.

Concentrations (ppm) in Some Key Compartments by Zones at the End of Two Years *

	ZONES			
Compartments	1	2	3	4
	Average Lead Concentrations (ppm)			
Plants & Litter	.08	.01	.0004	.63
Herbivores	5.23	.74	.05	16.21
Carnivores	3.13	.43	.03	10.22
Pavement	.0007**	.00008**		.0003**
Soil	.045	.006	.0003	.46
Drainage Water	.08	.01	.0007	.24
Soil Sink	1.09	.14	.008	10.87

* The above values represent a two-year average

** Results in g/m^2

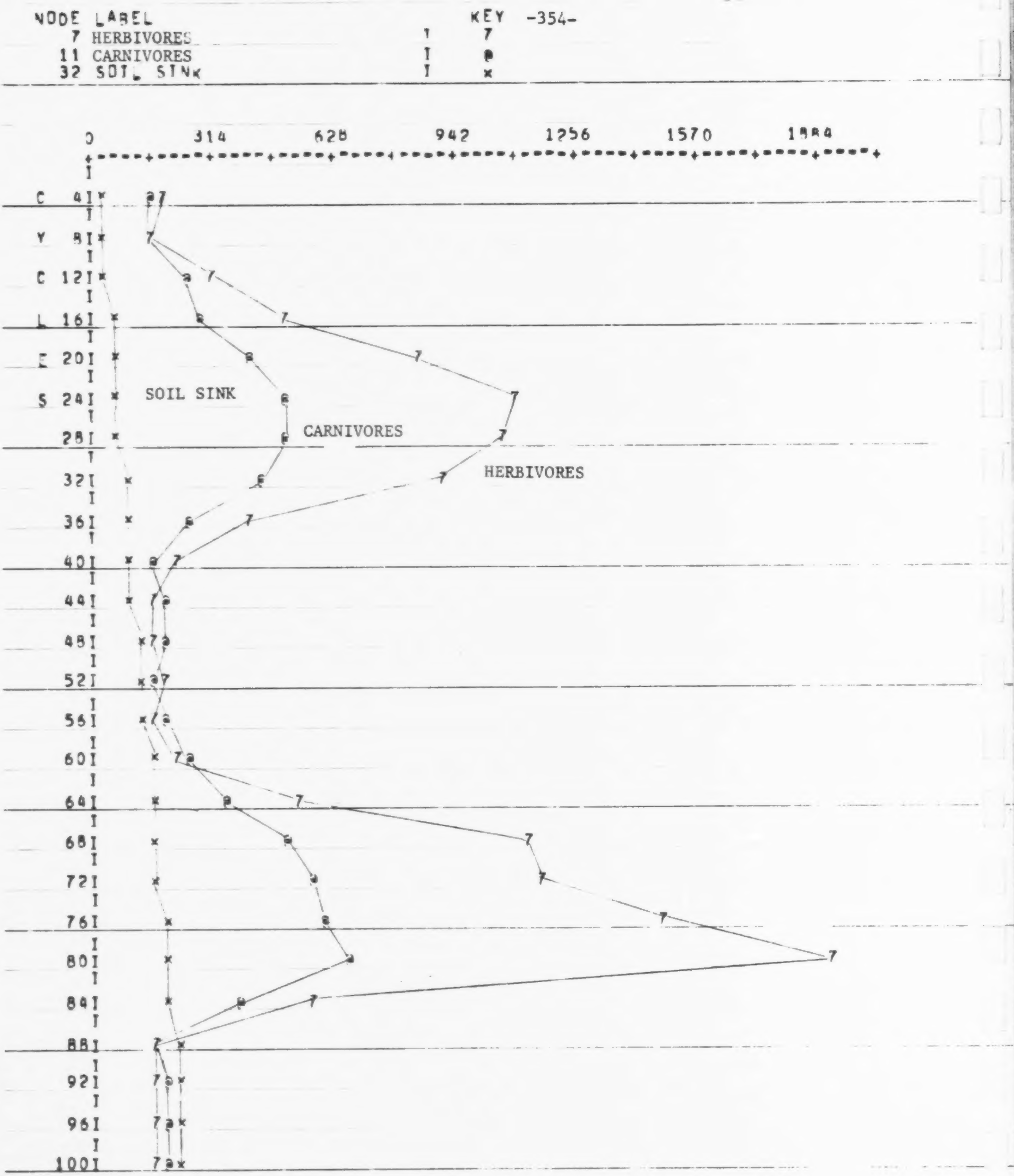


Figure 113. Expanded Network Output: Plot of Herbivores Carnivores, Soil Sinks (Zone I)

```
NODE LABEL                          KEY
   3 PLANTS + LITTER           I     3
  18 SOIL                      I     B
  22 SOIL WATER (DRAINAGE)     I     F
```

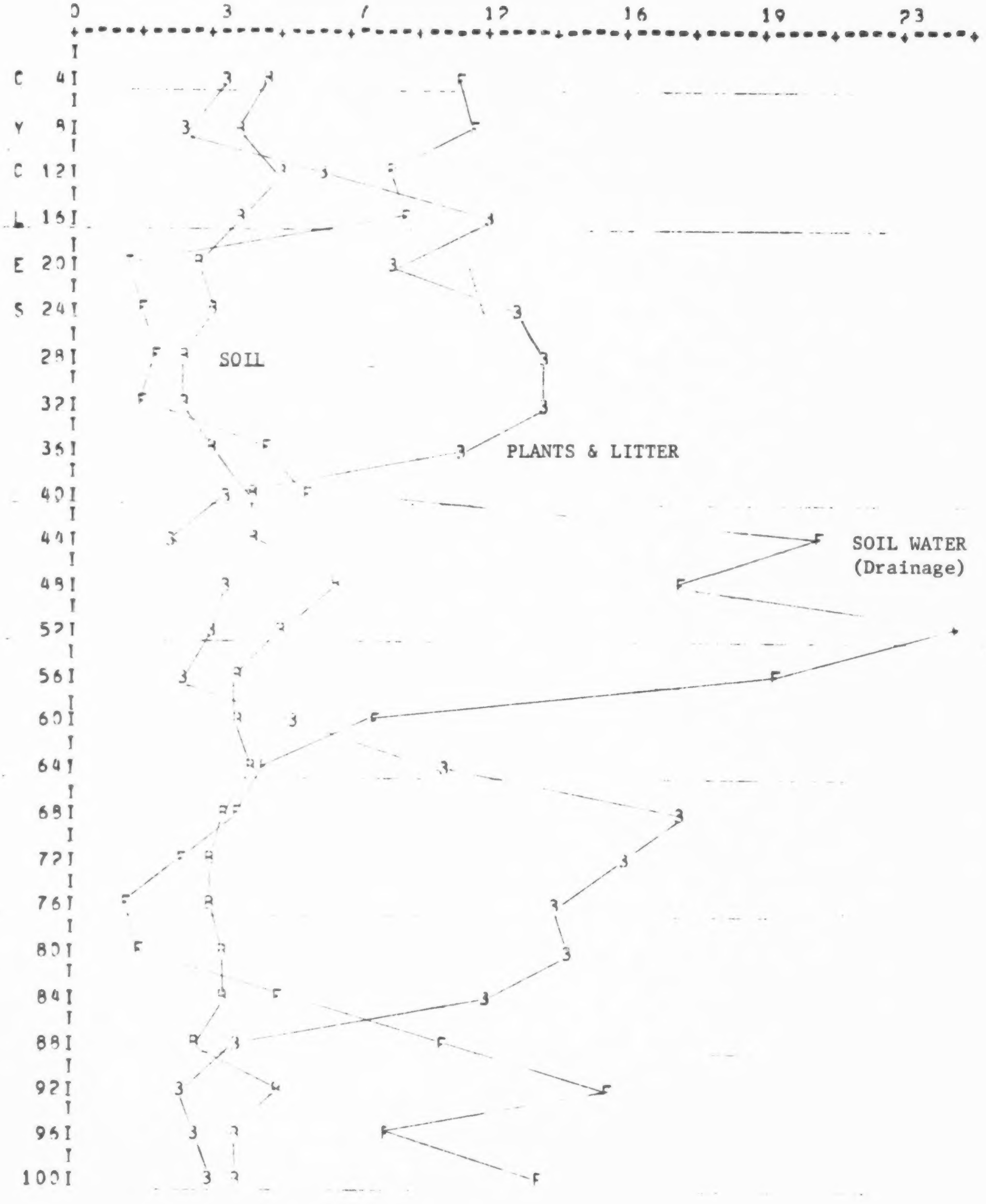


Figure 114. Expanded Network Output: Plot of Plants and Litter, Soil, Soil Water (Zone I)

```
NODE LABEL                                  KEY
   4 PLANTS + LITTER                     II   4
  19 SOIL                                II   C
  23 SOIL WATER (DRAINAGE)               II   G
```

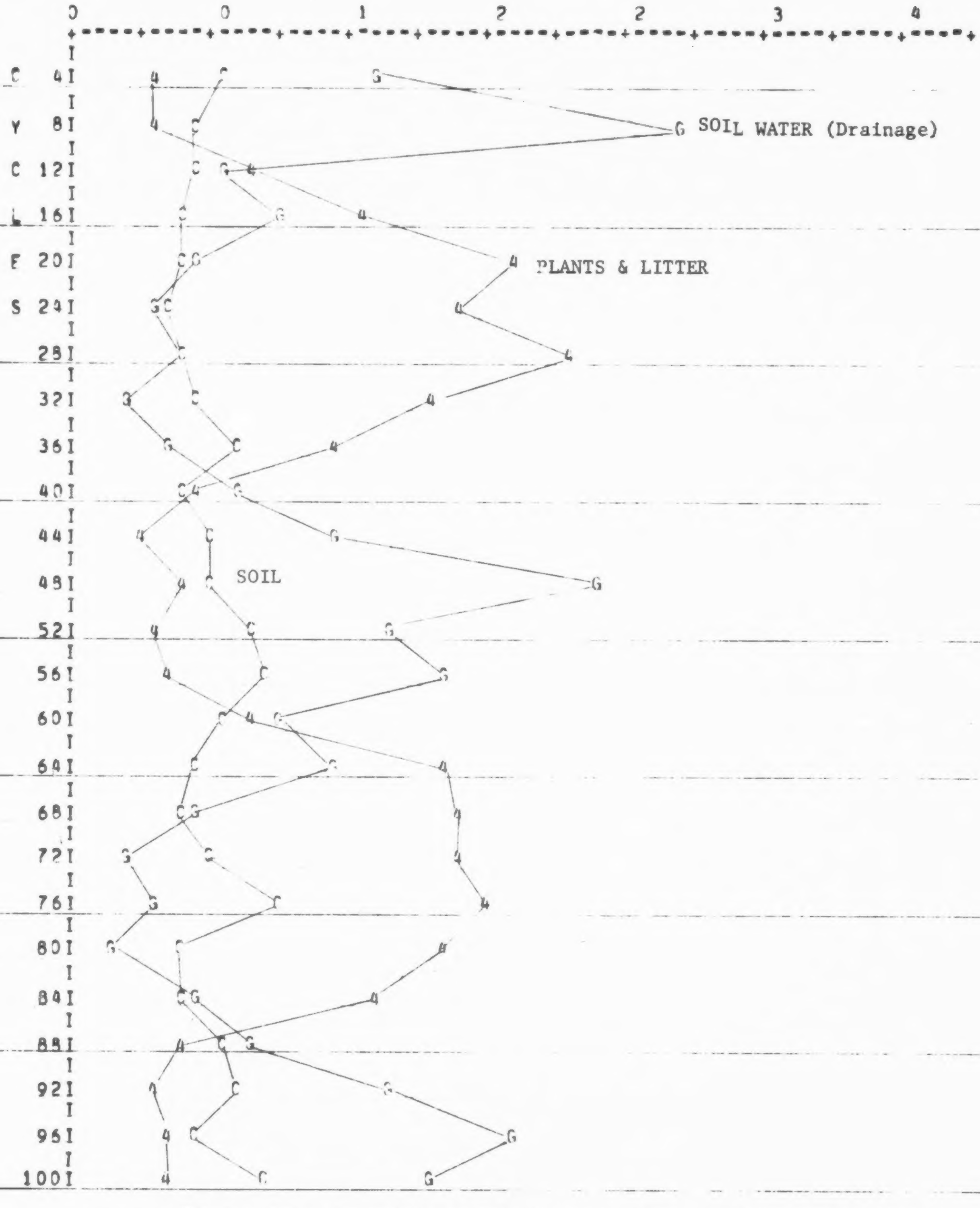


Figure 115. Expanded Network Output: Plot of Plants and Litter, Soil, Soil Water (Zone II)

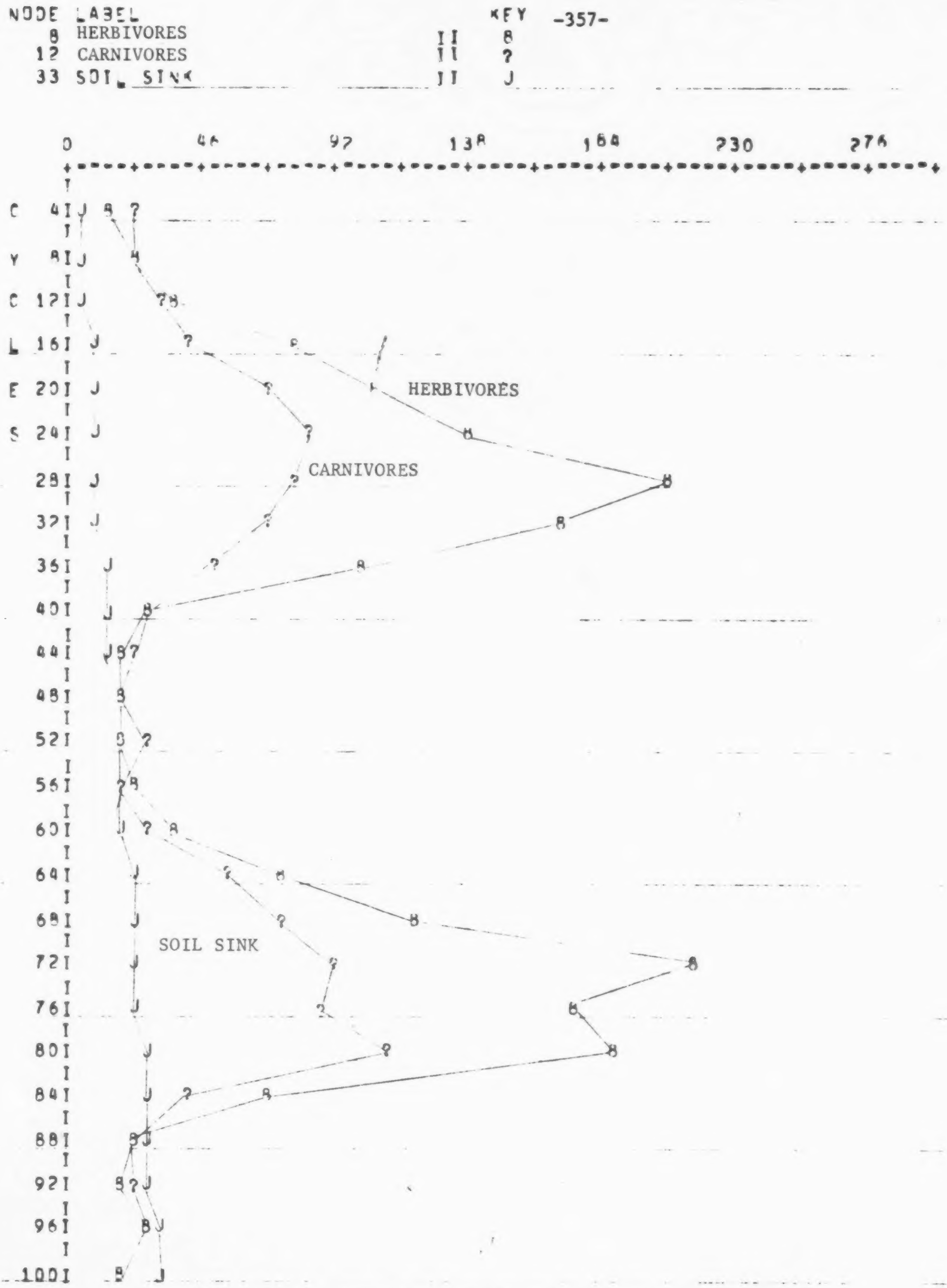


Figure 116. Expanded Network Output: Plot of Herbivores, Carnivores, Soil Sinks (Zone II)

```
NODE LABEL                           KEY
   2 ATMOSPHERE                       2
   3 PLANTS + LITTER            I     3
```

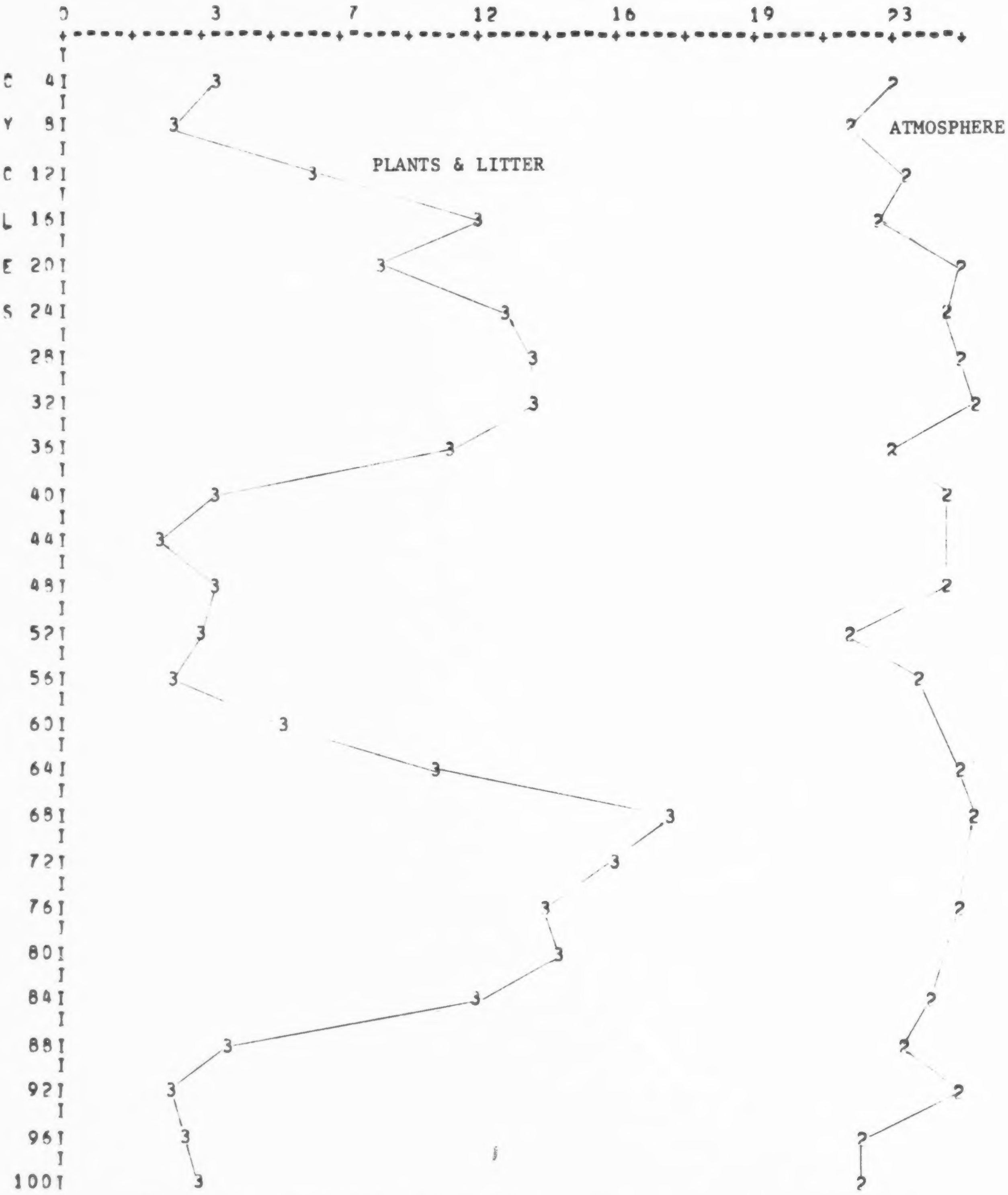


Figure 117. Expanded Network Output: Plot of Atmosphere, Plants and Litter (Zone I)

```
NODE LABEL                          KEY
  31 EXIT PLANT EXPORT               +
  32 SOIL SINK                 I     x
  33 SOIL SINK                 II    J
```

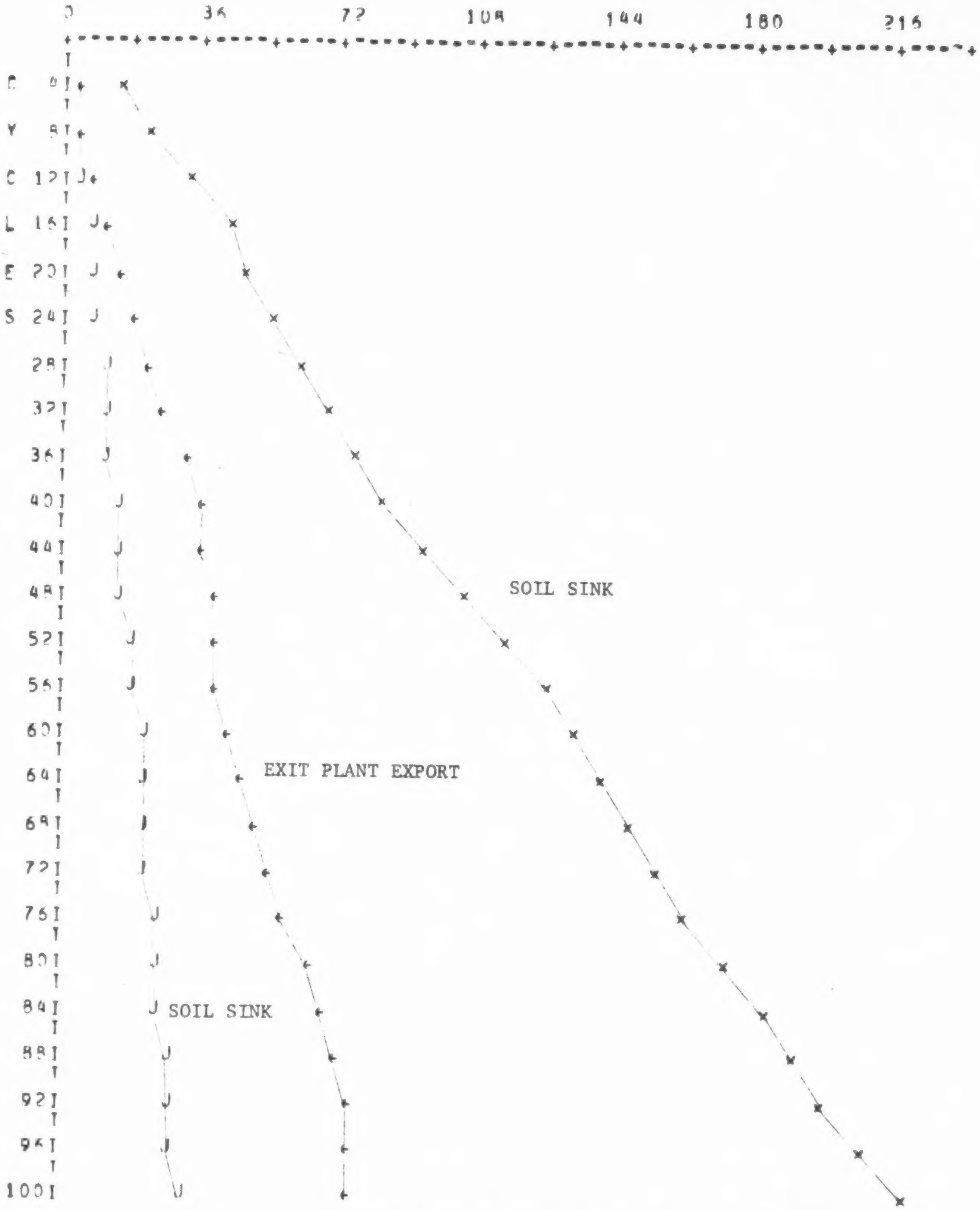


Figure 118. Expanded Network Output: Plot of Soil Sinks (Zones I and II)

further refinement of the model and input data, the future concentration of lead can be predicted.

It is felt that a further breakdown of the plants, carnivores, and herbivores could be useful. Also, it was initially assumed that the environment was clean to start with (no lead at all). It appears desirable to provide for an initial amount of lead in certain compartments such as soil.

Concentration results as shown in Table 44 compare favorably with estimated yearly increases found in the ecosystem for most compartments. However, the predicted plant and litter concentrations shown in Table 44, as well as the soil sink concentrations, appear to be considerably lower than those increases normally found. Parameter changes can be made when more field data are available.

B. Dynamic Model of Lead Flow in the Body

1. Introduction

A mathematical model has been developed to study the movement of lead in the body. Conceptually, the model is applicable to all mammals and most heavy metals. In this study, the main emphasis will be on lead in animals.

The model divides the body into a set of organs and relates the transfer of lead between these organs. This is based on two major assumptions: 1) the transfer of lead between organs is accomplished via the blood: and 2) the transfer of lead follows a first-order law. Two types of input are considered: the lead from air via respiration and the lead ingested with food and water. All other modes of input are neglected.

Three types of outputs are also considered. These are the lead output with urine, feces, and lead exhaled. Other possible outputs such as perspiration or hair removal are neglected.

Figure 119 shows the major lead flows for a given number of organs. A separation of the function of an organ from its physical entity leads to the conceptual model shown in Figure 120. Thus, the output of lead in the urine is assumed proportional to the blood concentration rather than the concentration of lead in the tissue of the kidney. The same applies to the G. I. tract and lead output in the feces.

2. Mathematical Model

While conceptually any number of organs can be incorporated into the model, only six are included in the formulation. These correspond to the organs examined in the experiment that is being presently performed on rats. Let:

KI, L, BO, M, SP, BL represent the amount of lead respectively in the Kidney, Liver, Bone, Muscle, Spleen, and Blood.

T represent the amount of lead in all other tissues, organs, and sinks not included in the model. The inclusion of this conceptual organ allows the conservation of equilibrium in the whole system.

UR, FE represent the amount of lead respectively in the urine and feces.

λ_i represent the rate constant for the transfer of lead from organ i to the blood.

λ^1_i represent the rate constant for the transfer of lead from the blood to organ i.

λ^1_u, λ^1_f represent respectively the rate constant for the transfer of lead from the blood to the urine and feces.

R_{fd} represent the rate input of lead to the body from food and water.

R^1_{fd}, R^1_{air} represent the rate input of lead to the blood from food and air, respectively.

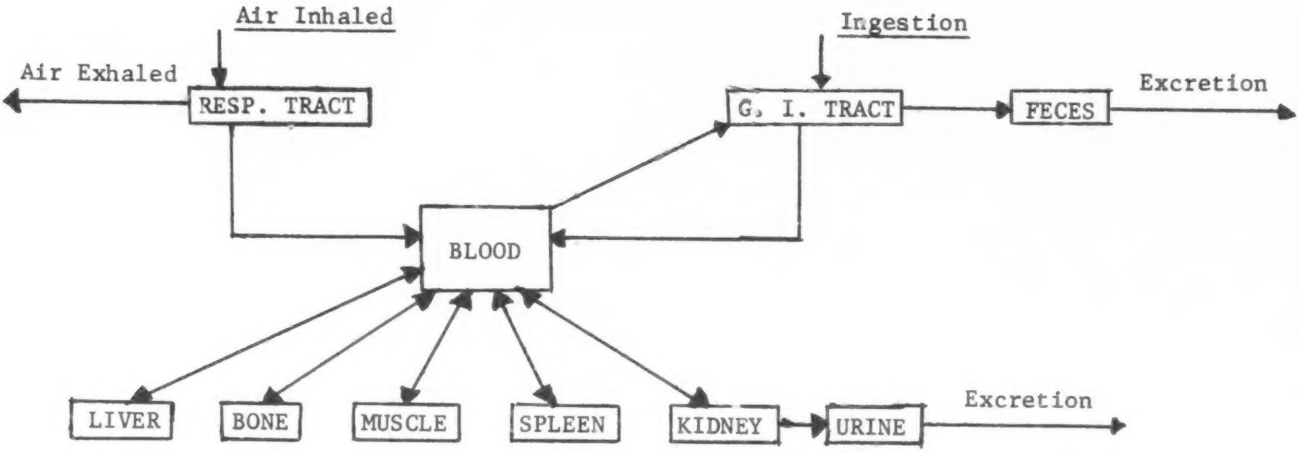

Air Inhaled
Air Exhaled
RESP. TRACT
Ingestion
G. I. TRACT
FECES
Excretion
BLOOD
LIVER
BONE
MUSCLE
SPLEEN
KIDNEY
URINE
Excretion

Figure 119. Physical Flow

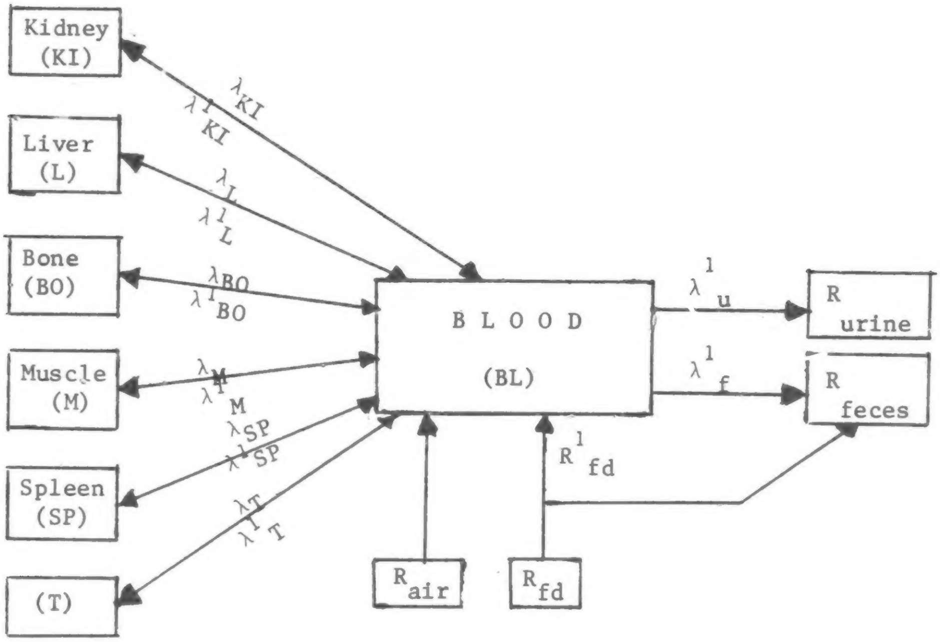

Kidney (KI)
Liver (L)
Bone (BO)
Muscle (M)
Spleen (SP)
(T)
λ_{KI}
λ^1_{KI}
λ_L
λ^1_L
λ_{BO}
λ^1_{BO}
λ_M
λ^1_M
λ_{SP}
λ^1_{SP}
λ_T
λ^1_T
B L O O D
(BL)
λ^1_u
λ^1_f
R urine
R feces
R^1_{fd}
R_{air}
R_{fd}

Figure 120. Conceptual Model

BR represent breathing rate.

CA represent concentration of lead in air.

X_A represent the fraction of lead from air absorbed into the blood.

IR_n represent the ingestion rate of food type n.

CE_n represent the concentration of lead in food type n.

X_n represent the fraction of lead absorbed into the blood from food type n.

Then,

the input rates to the system

$$R_{air} = (BR)\ (CA)\ (X_A)$$

$$R^1_{fd} = \sum_{n=1}^{N} (IR_n)\ (CE_n)\ (X_n)$$

the output rates from the system

$$FE = \lambda^1(BL) + (R_{fd} - R_{fd})$$

$$= \lambda^1_f(BL) + \sum_{n=1}^{N}(CE_n)\ (I_n)\ (1 - X_n)$$

$$UR = \lambda^1_u(BL)$$

the equilibrium equations

$$\frac{d(KI)}{dt} = -\lambda_{KI}(KI) + \lambda^1_{KI}(BL)$$

$$\frac{d(L)}{dt} = -\lambda_L(L) + \lambda^1_L(BL)$$

$$\frac{d(BO)}{dt} = -\lambda_{BO}(BO) + \lambda^1_{BO}(BL)$$

$$\frac{d(M)}{dt} = -\lambda_M(M) + \lambda^1_M(BL)$$

$$\frac{d(SP)}{dt} = -\lambda_{SP}(SP) + \lambda^1_{SP}(BL)$$

$$\frac{d(T)}{dt} = -\lambda_T(T) + \lambda^1{}_T(BL)$$

$$\frac{d(BL)}{dt} = -\frac{d(KI)}{dt} - \frac{d(L)}{dt} - \frac{d(BO)}{dt} - \frac{d(M)}{dt} - \frac{d(SP)}{dt} - \frac{d(T)}{dt}$$
$$+ R_{air} + R^1{}_{fd} - \lambda^1{}_u(BL) - \lambda^1{}_f(BL)$$

The above system of equations satisfies the dynamic equilibrium conditions at each organ and for the system as a whole. Relative to the assumptions of the model, a given input and the rate constants λ_i and $\lambda^1{}_i$, the model will yield the concentration of lead in the various organs at any given time. Thus, it can be used to predict the accumulation of lead in critical organs relative to a given input.

It is hypothesized that for a given species, a given organ and a given direction of flow, a mean rate constant denoted by λ exists. While conceptually λ will also depend on other factors such as the diet, the biological variables within a population and the chemical form of lead, to name a few, at this stage of the investigation these are neglected.

3. Experimentation

An experiment with rats described in another section of this report was designed to be used in conjunction with this model. In this respect the experiment has two purposes: 1) to determine the rate constants described earlier for a given species, namely rats (this will be achieved by monitoring the change of concentration in various organs with time relative to a given input; 2) to validate the proposed model or suggest modifications so that the flow of lead in the body can be described adequately. As of this date, the experiment is still in progress and the results are not available to test the model.

A survey of the existing literature on the topic provided no data that can be used to find the necessary parameters of the model. The "Report of Committee II on Permissible Dose for Internal Radiation - 1959", published for "The International Commission on Radiological Protection", lists the permissible doses of lead for some organs of the human body. The data published in this report were interpreted loosely to provide a rough approximation for the rate constants of three organs listed for an "average" man. For the purposes of illustration, two models for man were used. They will be referred to as Man-Model I and Man-Model II. They are shown respectively in Figures 121 and 122.

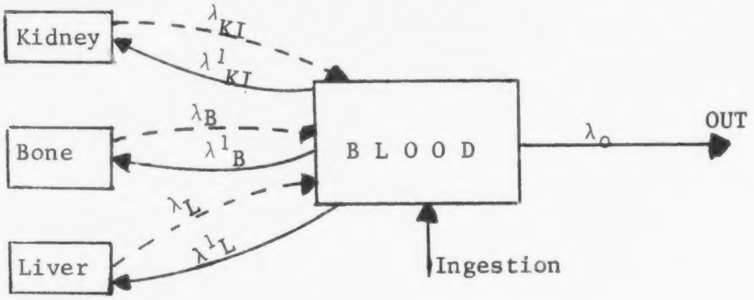

Kidney
λ_{KI}
λ^1_{KI}
Bone
λ_B
λ^1_B
Liver
λ_L
λ^1_L
B L O O D
λ_o
OUT
Ingestion

Figure 121. Man-Model I

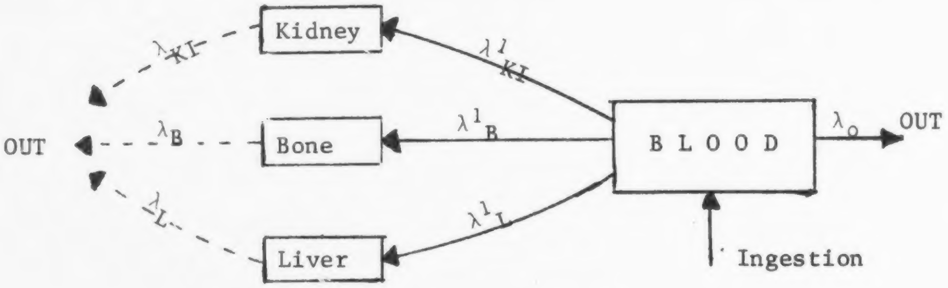

Kidney
λ_{KI}
λ^1_{KI}
OUT
λ_B
Bone
λ^1_B
B L O O D
λ_o
OUT
λ_L
Liver
λ^1_L
Ingestion

Figure 122. Man-Model II

In each of these two models, it was assumed that the body consists of only four organs and that lead input is only via ingestion. The data used in these models are given in Table 45.

The IBM CSMP program was used on the IBM 360/75 computer of the University of Illinois. Each of the two models was run for a period of 2000 weeks, starting with zero concentration. The results are reported in Table 46 and Table 47. The plot of the concentration versus time is shown in Figure 123 and Figure 124.

Clearly no inferences can be made from the results due to the assumptions made on the data used and the simplified models. Nevertheless, they are presented because they illustrate that the model may be satisfactory for describing the dynamic flow of lead in the body.

C. Plant Modeling

1. Introduction

A model is being developed to describe the movements and distribution of heavy metals through the Air-Soil-Water Plant System. The model represents the plant environment interaction for the various species of plants found within the watershed ecosystem and elsewhere.

The objective of the model is to determine the concentration of a heavy metal (lead) at any time (t) in the various tissues of the plant, given exposure to the metal in the input elements.

Data for development of the model are being derived from uptake experiments with species typical of the ecosystem. Most of these studies are currently in progress.

Table 45

Biological Parameters for Lead in Human Body

ELEMENT	Biological half life (days)	Rate Constant (weeks^{-1})	Fraction from Blood to Organ of reference	Mass of Organ (grams)
Total Body	1.46×10^3	$\lambda_O = 0.0033$		
Bone	3.65×10^3	$\lambda_B = 0.0013$	$\lambda^1{}_B = 0.28$	7×10^3
Liver	1947	$\lambda_L = 0.0025$	$\lambda^1{}_L = 0.08$	1.7×10^3
Kidney	531	$\lambda_{KI} = 0.009$	$\lambda^1{}_{KI} = 0.14$	300

Ingestion to Blood — Input to Blood = 0.0002 grams/week

Table 46

Man-Model I. Projected Accumulation

Organ	Accumulation after 2000 weeks - grams	Concentration after 2000 weeks - ppm
Bone	3.06×10^{-1}	43.7
Liver	5.41×10^{-2}	31.8
Kidney	2.96×10^{-2}	98.7
Blood	1.98×10^{-3}	0.4

Table 47

Man-Model II. Projected Accumulation

Organ	Accumulation after 2000 weeks - grams	Concentration after 2000 weeks - ppm
Bone	7.92×10^{-2}	11.3
Liver	1.26×10^{-2}	7.4
Kidney	6.18×10^{-3}	20.7
Blood	3.97×10^{-4}	0.07

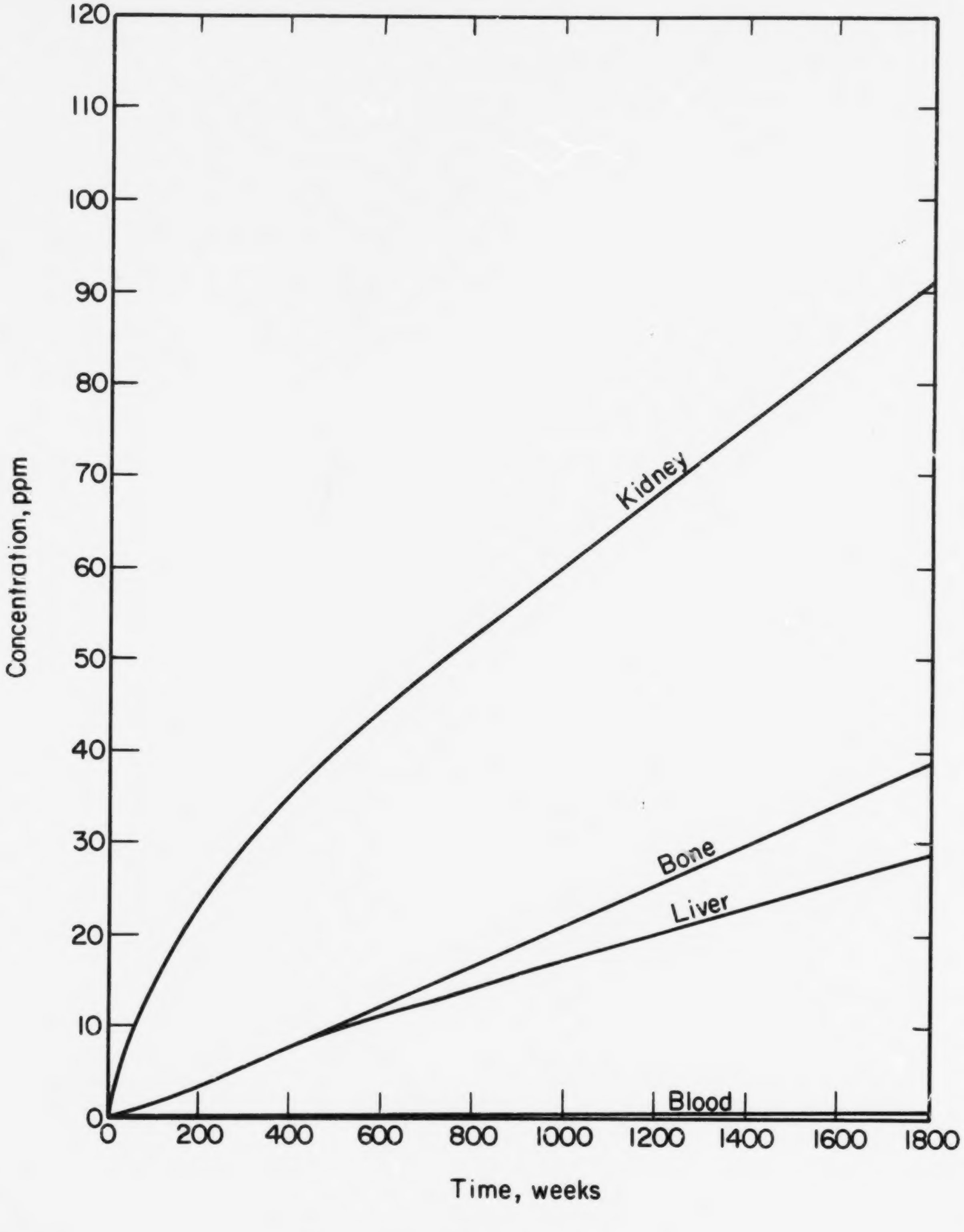


Figure 123 Man Model I

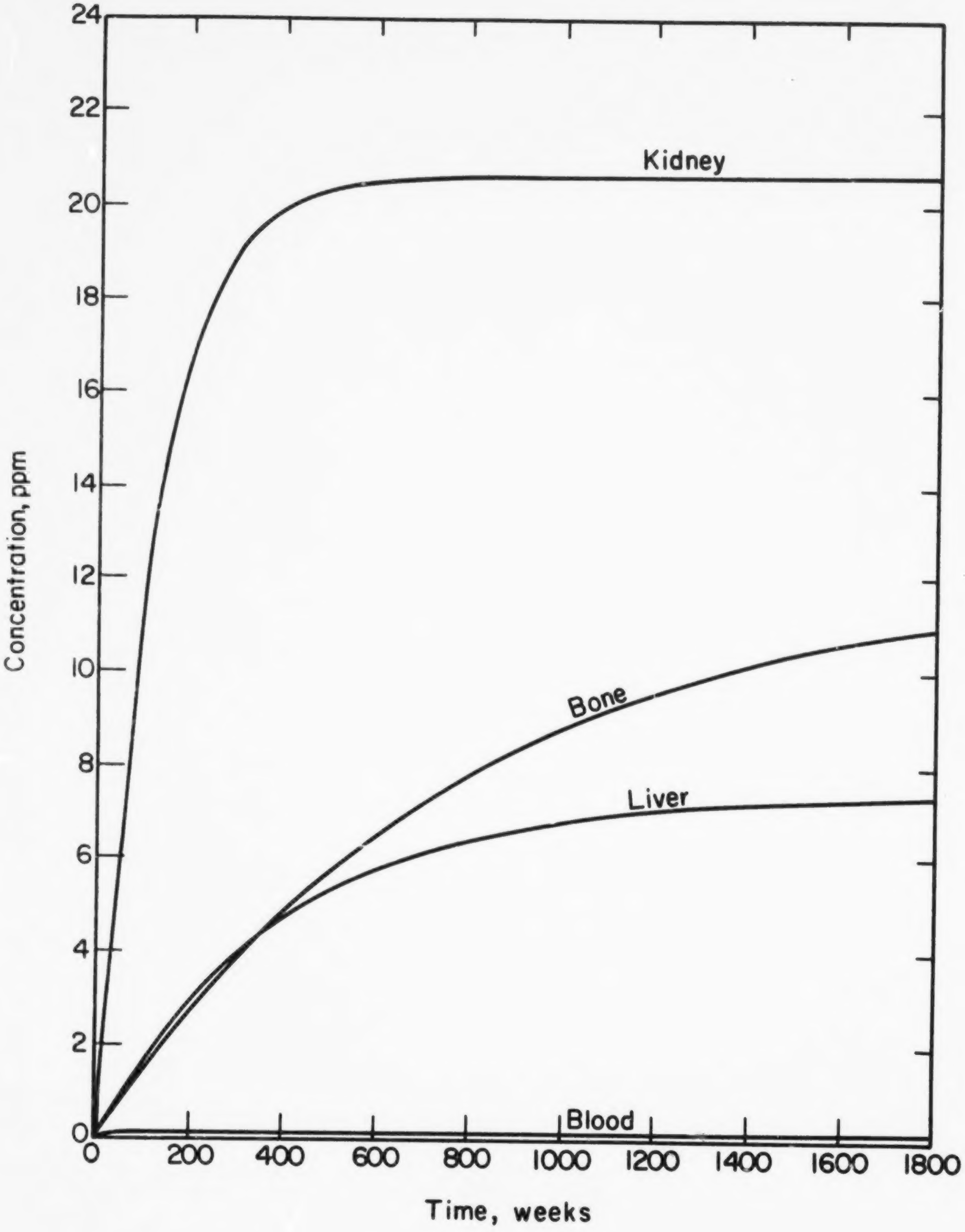


Figure 124 Man-Model II

2. Components of the Model

Three types of components may be distinguished in the system:

- a. Input elements
  - 1. Air
  - 2. Soil (soil water)
- b. Output elements
  - 1. Soil
  - 2. Litter
- c. Plant elements
  - 1. Root
  - 2. Stem
  - 3. Fruit
  - 4. Leaves

A more detailed breakdown of all types of elements can easily be added to the model.

d. Rate transmission constants

Two types of rate transmission constants have been defined initially, one for each of the two types of flow found in the plant-environment system:

1. K_{ij}: This rate transmission constant is associated with a flow of lead from element i to element j caused by a buildup of lead concentration in element i. The presence of lead in element i generates flow to element j, K_{ij} is associated with such flow and is assumed to remain constant for any lead concentration in element i.

2. L_{ij}: This rate transmission constant is associated with a flow of lead from element i to element j caused by various processes such as harvesting, leaf fall, rain washing of leaves, insect damage, etc. In general, L_{ij} is not associated with flows generated by a buildup of lead concentration in element i.

At this stage, experiments are being conducted in order to determine the values of K_{ij} for certain plant species. Only qualitative estimations can be made at present for L_{ij} components.

3. Assumptions of the Model

a. Conservation of flow holds for each model element:
Flow Rate in = Flow Rate Out + Storage Rate.

b. The rate of flow of lead from element i to element j is proportional to the lead concentration in element i. The proportionality constant is equal to the rate transmission constant K_{ij}, or L_{ij}. In equation form we would have:

$$\text{Flow from element } i \text{ to } j = K_{ij}[Pb]_i, \text{ or}$$
$$= L_{ij}[Pb]_i$$

c. In relation to K_{ij} two important assumptions are made. First, that K_{ij} remains constant during the growing season, and then drops to zero after the growing season but regains its previous value in the following growing season, as depicted by Figure 125. Second, it is assumed that K_{ij} remains constant for different lead concentrations in element i.

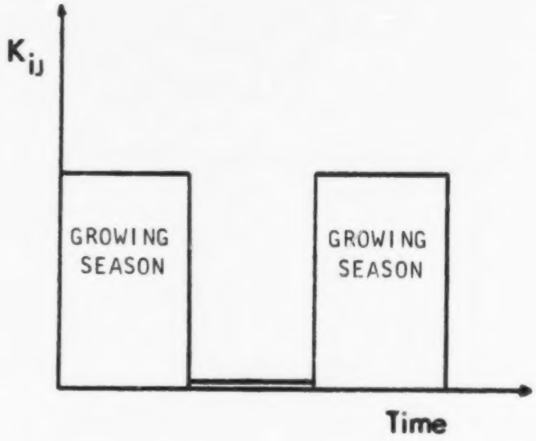


Figure 125.

Variation with Time of K_{ij}

d. L_{ij} includes under the same grouping several concepts which have in common the fact that they represent flow paths of lead in the system not caused by a concentration buildup of the metal in element i. For harvesting and leaf fall in autumn, Figure 126 and Figure 127, respectively, present how L_{ij} may change with time.

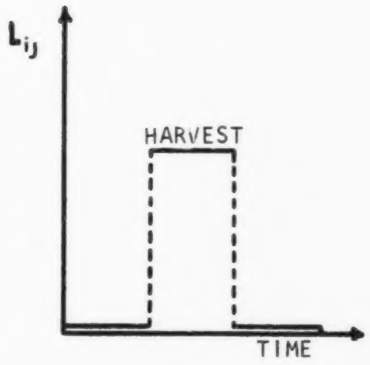


Figure 126.
Seasonal Variations

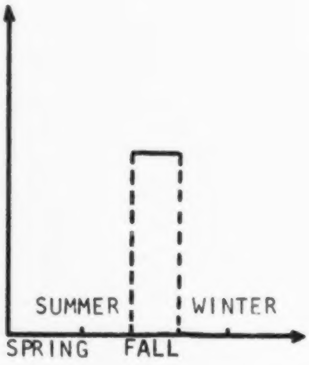


Figure 127.
Seasonal Variations

It is recognized that many of the assumptions stated above are not valid for an ultimate description of heavy metal movement in the plant system. However these assumptions are made to provide a starting point for development of the plant system model.

4. Interconnection Matrices

a. K_{ij} type flow

All possible flows from element j are marked with X.

FROM \ TO	SOIL	ROOT	STEM	FRUIT	LEAF	LITTER
AIR			X	X	X	X
SOIL		X				
ROOT	X		X			
STEM		X		X	X	X
FRUIT			X			X
LEAVES			X			X
LITTER	X					

Flow path AIR-SOIL is not included since these two elements are considered as inputs independent of each other and their relationship is not the interest of this study.

b. L_{ij} type flow

FROM \ TO	SOIL	LITTER
STEM		X
FRUIT		X
LEAVES		X
LITTER	X	

5. Linear Graph of the Plant Model

Some of the possible flows marked on the interconnection matrices table are considered to be negligible and consequently they are not included in the model (Figure 128).

6. Equations

It is possible to set up a conservation of flow equation for each of the output and plant elements included in the model.

The conservation of flow equation is:

Rate in = Rate out + Storage Rate;

and in general, for model element i we have:

$$\frac{d\ [i]}{dt} = \sum_{a}^{j} K_{ji}\ [j] + \sum_{a}^{j} L_{ji}\ [j] - \sum_{b}^{l} K_{il}\ [i] - \sum_{b}^{l} L_{il}\ [i] \qquad (1)$$

a = summation over all incoming branches from elements j

b = summation over all outgoing branches to elements l.

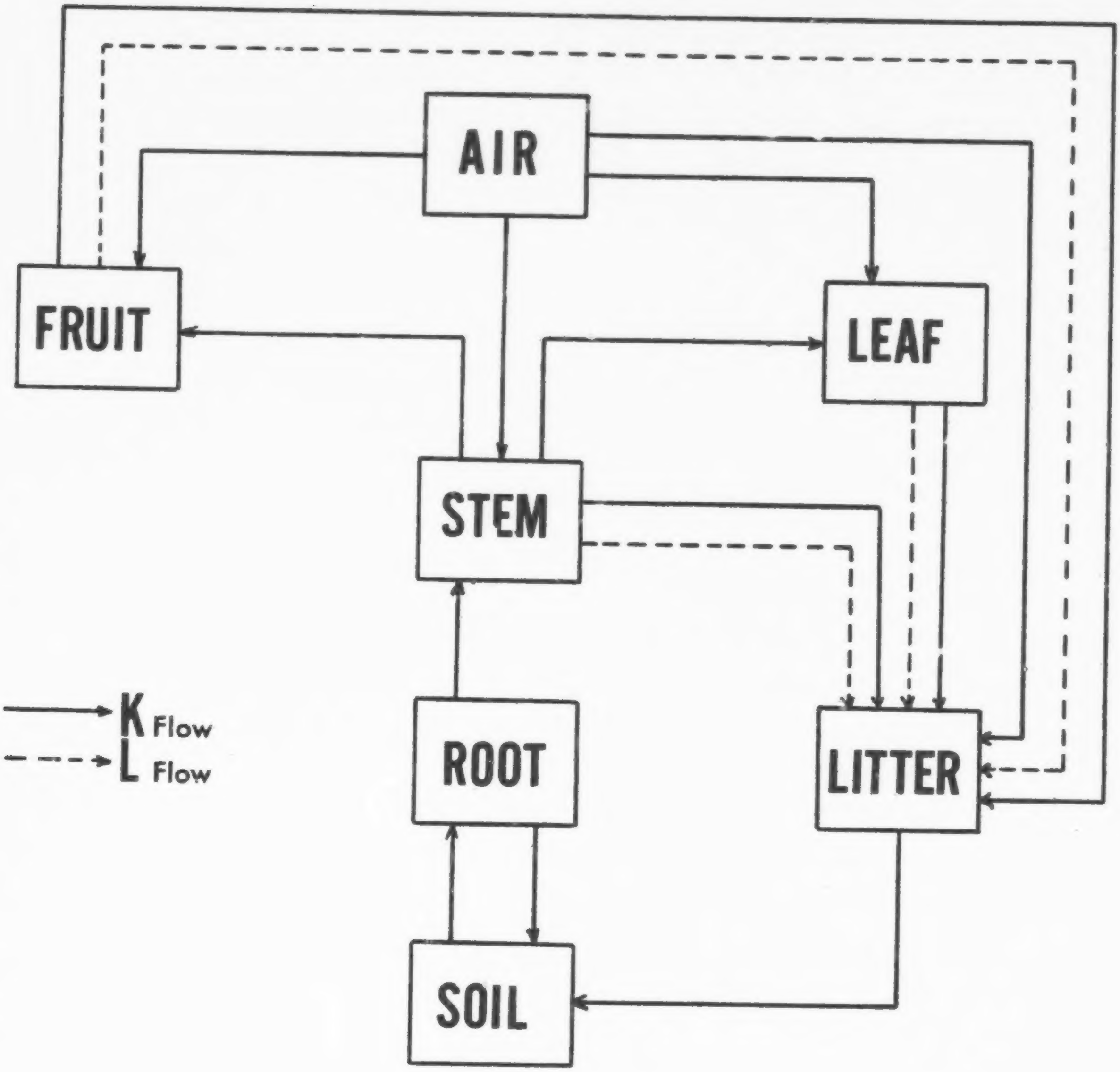

AIR
FRUIT
LEAF
STEM
ROOT
LITTER
SOIL
K Flow
L Flow

Figure 128. Plant Model

Equation (1) can be applied to each of the output and plant elements on the model. A system of simultaneous differential equations will thus be formed. The solution is carried out by using the program 360 Continuous System Modeling Program (360/CSMP) available in IBM 360 computers.

The solution of the equations will yield the lead concentrations in the model elements as a function of time for the given input concentrations in AIR and SOIL.

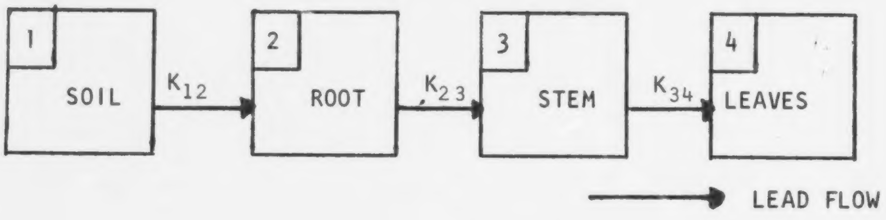
7. Example of the Model

A simplified plant model was constructed using some of the elements named in Figure 128.

The following elements were included in the model: SOIL, ROOT, STEM and LEAVES. These elements were considered because of the availability of experimental data to calculate the respective K_{ij} transmission constants.

a. Determination of K_{ij} Constants

In order to calculate the different K_{ij}'s, the following model was used:



Applying equation (1):

$$\dot{R} = K_{12}SL - K_{23}\ R \tag{2a}$$

$$\dot{SM} = K_{23}R - K_{34}\ SM \tag{2b}$$

$$\dot{L} = K_{34}\ SM \tag{2c}$$

where:

. = first derivative with respect to time

R = Lead in Root (grams)

SM = Lead in Stem (grams)

L = Lead in Leaves (grams)

Data were available for lead concentrations in the root, stem, and leaves of cottonwood (Populus deltoides) at three times during its growing season: 0,30,and 60 days. The initial soil lead concentration was 75 ppm.

From the data it was possible to calculate $\dot{R}$, $\dot{SM}$, and $\dot{L}$, and their corresponding R, SM,and L at time 30 days. Substituting these values into equation set (2) the following values for K_{ij} were obtained:

$K_{12} = 3.5 \times 10^{-6}$	(Days $^{-1}$)
$K_{23} = 8.36 \times 10^{-6}$	(Days $^{-1}$)
$K_{34} = 15.5 \times 10^{-6}$	(Days $^{-1}$)

The model was constructed using equation set (2) and was implemented in CSMP/360 and run on the IBM 360/75 computer.

Equation set (2) describes the behavior of the plant during the growing period (26 weeks). At the end of the growing period the flow from soil to root stops and the concentration of lead at the root and stem remains constant until the next growing season as may be seen in Figures 129 and 130. It is assumed that when the growing season ends, the leaves begin falling and thus the total lead remaining in leaves on the plant decreases to a negligible amount similar to the concentration plot shown in Figure 131. This is a L_{ij} type flow and the value of the rate transmission constant was determined such that 13 weeks after the growing season ends the number of leaves on the plant is negligible.

The concentrations at 30 and 60 days (shown in Figures 129, 130, and 131) using the model were very similar to the experimental data concentrations. Initial concentrations of lead in the plant elements (from the experimental data) were:

$R = 4.6 \text{ X } 10^{-6}$ grams
$SM = 3.4 \text{ X } 10^{-6}$ grams
$L = 1.8 \text{ X } 10^{-6}$ grams

Additional experimental work is in progress to provide better data for the model. These experiments will involve a range of soil-lead concentrations between 0 and 80 ppm as well as plant-lead concentrations at two-week intervals throughout a 12-week growing season.

D. Atmospheric Transport of Lead-Containing Particles

A mathematical model is being developed which will determine the atmospheric concentration and the ground deposition rate of lead particles in the seventy-six-square-mile region under consideration at the University of Illinois. The primary sources of lead are (1) a compact rectangular

```
                                MINIMUM                         RPPM    VERSUS TIME
                               1.7037E-06
 TIME (weeks)        RPPM          I
 0.0                 1.7037E-06    +
 1.0000E 00          4.2510E-06    --+
 2.0000E 00          6.6535E-06    -----+
 3.0000E 00          8.9194E-06    --------+
 4.0000E 00          1.1056E-05    ----------+
 5.0000E 00          1.3072E-05    -------------+
 6.0000E 00          1.4973E-05    ---------------+
 7.0000E 00          1.6766E-05    -----------------+
 8.0000E 00          1.8457E-05    -------------------+
 9.0000E 00          2.0052E-05    ---------------------+
 1.0000E 01          2.1557E-05    -----------------------+
 1.1000E 01          2.2375E-05    ------------------------+
 1.2000E 01          2.4314E-05    --------------------------+
 1.3000E 01          2.5576E-05    ---------------------------+
 1.4000E 01          2.6766E-05    -----------------------------+
 1.5000E 01          2.7880E-05    ------------------------------+
 1.6000E 01          2.8948E-05    -------------------------------+
 1.7000E 01          2.9946E-05    ---------------------------------+
 1.8000E 01          3.0888E-05    ----------------------------------+
 1.9000E 01          3.1777E-05    -----------------------------------+
 2.0000E 01          3.2615E-05    ------------------------------------+
 2.1000E 01          3.3405E-05    -------------------------------------+
 2.2000E 01          3.4150E-05    --------------------------------------+
 2.3000E 01          3.4853E-05    --------------------------------------+
 2.4000E 01          3.5516E-05    ---------------------------------------+
 2.5000E 01          3.6142E-05    ----------------------------------------+
 2.6000E 01          3.6684E-05    -----------------------------------------+
 2.7000E 01          3.6684E-05    -----------------------------------------+
 2.8000E 01          3.6684E-05    -----------------------------------------+
 2.9000E 01          3.6684E-05    -----------------------------------------+
 3.0000E 01          3.6684E-05    -----------------------------------------+
 3.1000E 01          3.6684E-05    -----------------------------------------+
 3.2000E 01          3.6684E-05    -----------------------------------------+
 3.3000E 01          3.6684E-05    -----------------------------------------+
 3.4000E 01          3.6684E-05    -----------------------------------------+
 3.5000E 01          3.6684E-05    -----------------------------------------+
 3.6000E 01          3.6684E-05    -----------------------------------------+
 3.7000E 01          3.6684E-05    -----------------------------------------+
 3.8000E 01          3.6684E-05    -----------------------------------------+
 3.9000E 01          3.6684E-05    -----------------------------------------+
 4.0000E 01          3.6684E-05    -----------------------------------------+
 4.1000E 01          3.6684E-05    -----------------------------------------+
 4.2000E 01          3.6684E-05    -----------------------------------------+
 4.3000E 01          3.6684E-05    -----------------------------------------+
 4.4000E 01          3.6684E-05    -----------------------------------------+
 4.5000E 01          3.6684E-05    -----------------------------------------+
 4.6000E 01          3.6684E-05    -----------------------------------------+
 4.7000E 01          3.6684E-05    -----------------------------------------+
 4.8000E 01          3.6684E-05    -----------------------------------------+
 4.9000E 01          3.6684E-05    -----------------------------------------+
 5.0000E 01          3.6684E-05    -----------------------------------------+
```

Figure 129. Lead Concentration (ppm) in the Root

```
                                        MINIMUM                   SMPPM  VERSUS TIME
                                      1.6191E-06
 TIME  (weeks)       SMPPM                I
 0.0               1.6191E-06             +
 1.0000E 00        1.6674E-06             +
 2.0000E 00        1.8871E-06             +
 3.0000E 00        2.2505E-06             -+
 4.0000E 00        2.7334E-06             --+
 5.0000E 00        3.3145E-06             ---+
 6.0000E 00        3.9755E-06             ----+
 7.0000E 00        4.7000E-06             -----+
 8.0000E 00        5.4742E-06             -------+
 9.0000E 00        6.2858E-06             --------+
 1.0000E 01        7.1244E-06             ----------+
 1.1000E 01        7.9309E-06             -----------+
 1.2000E 01        8.8475E-06             -------------+
 1.3000E 01        9.7176E-06             --------------+
 1.4000E 01        1.0586E-05             ----------------+
 1.5000E 01        1.1447E-05             -----------------+
 1.6000E 01        1.2297E-05             -------------------+
 1.7000E 01        1.3133E-05             --------------------+
 1.8000E 01        1.3953E-05             ----------------------+
 1.9000E 01        1.4753E-05             -----------------------+
 2.0000E 01        1.5533E-05             -------------------------+
 2.1000E 01        1.6290E-05             --------------------------+
 2.2000E 01        1.7024E-05             ---------------------------+
 2.3000E 01        1.7734E-05             -----------------------------+
 2.4000E 01        1.8420E-05             ------------------------------+
 2.5000E 01        1.9082E-05             -------------------------------+
 2.6000E 01        1.9666E-05             --------------------------------+
 2.7000E 01        1.9666E-05             --------------------------------+
 2.8000E 01        1.9666E-05             --------------------------------+
 2.9000E 01        1.9666E-05             --------------------------------+
 3.0000E 01        1.9666E-05             --------------------------------+
 3.1000E 01        1.9666E-05             --------------------------------+
 3.2000E 01        1.9666E-05             --------------------------------+
 3.3000E 01        1.9666E-05             --------------------------------+
 3.4000E 01        1.9666E-05             --------------------------------+
 3.5000E 01        1.9666E-05             --------------------------------+
 3.6000E 01        1.9666E-05             --------------------------------+
 3.7000E 01        1.9666E-05             --------------------------------+
 3.8000E 01        1.9666E-05             --------------------------------+
 3.9000E 01        1.9666E-05             --------------------------------+
 4.0000E 01        1.9666E-05             --------------------------------+
 4.1000E 01        1.9666E-05             --------------------------------+
 4.2000E 01        1.9666E-05             --------------------------------+
 4.3000E 01        1.9666E-05             --------------------------------+
 4.4000E 01        1.9666E-05             --------------------------------+
 4.5000E 01        1.9666E-05             --------------------------------+
 4.6000E 01        1.9666E-05             --------------------------------+
 4.7000E 01        1.9666E-05             --------------------------------+
 4.8000E 01        1.9666E-05             --------------------------------+
 4.9000E 01        1.9666E-05             --------------------------------+
 5.0000E 01        1.9666E-05             --------------------------------+
```

Figure 130. Lead Concentration (ppm) in the Stem

```
                         MINIMUM                  LPPM   VERSUS TIME
                       -1.6695E-10
 TIME (weeks)    LPPM        I
0.0              1.3846E-06  +
1.0000E 00       1.6699E-06  +
2.0000E 00       1.9791E-06  +
3.0000E 00       2.3399E-06  -+
4.0000E 00       2.7750E-06  -+
5.0000E 00       3.3037E-06  -+
6.0000E 00       3.9415E-06  -+
7.0000E 00       4.7009E-06  --+
8.0000E 00       5.5919E-06  --+
9.0000E 00       6.6220E-06  --+
1.0000E 01       7.7369E-06  ---+
1.1000E 01       9.1205E-06  ----+
1.2000E 01       1.0595E-05  ----+
1.3000E 01       1.2222E-05  -----+
1.4000E 01       1.4001E-05  ------+
1.5000E 01       1.5932E-05  -------+
1.6000E 01       1.8013E-05  --------+
1.7000E 01       2.0242E-05  ---------+
1.8000E 01       2.2616E-05  ----------+
1.9000E 01       2.5132E-05  -----------+
2.0000E 01       2.7786E-05  ------------+
2.1000E 01       3.0575E-05  -------------+
2.2000E 01       3.3495E-05  ---------------+
2.3000E 01       3.6542E-05  ----------------+
2.4000E 01       3.9710E-05  -----------------+
2.5000E 01       4.2997E-05  -------------------+
2.6000E 01       2.6947E-05  ------------+
2.7000E 01       1.0604E-05  ----+
2.8000E 01       4.1724E-06  -+
2.9000E 01       1.6418E-06  +
3.0000E 01       6.4604E-07  +
3.1000E 01       2.5421E-07  +
3.2000E 01       1.0003E-07  +
3.3000E 01       3.9361E-08  +
3.4000E 01       1.5488E-08  +
3.5000E 01       6.0944E-09  +
3.6000E 01       2.3981E-09  +
3.7000E 01       9.4363E-10  +
3.8000E 01       3.7131E-10  +
3.9000E 01      -6.6657E-11  +
4.0000E 01      -6.6657E-11  +
4.1000E 01      -6.6657E-11  +
4.2000E 01      -6.6657E-11  +
4.3000E 01      -6.6657E-11  +
4.4000E 01      -6.6657E-11  +
4.5000E 01      -6.6657E-11  +
4.6000E 01      -6.6657E-11  +
4.7000E 01      -6.6657E-11  +
4.8000E 01      -6.6657E-11  +
4.9000E 01      -6.6657E-11  +
5.0000E 01      -6.6657E-11  +
```

Figure 131. Lead Concentration (ppm) in the Leaves

POTENTIAL WIDE-INTEREST REPORT

WARNING TO EVALUATORS FROM INPUT

DOCUMENT WILL REPRODUCE POORLY

DECISION BY EVALUATORS:

1. Accepted as wide-interest report ______________________ Signature

2. Accepted as regular report ______________________ Signature

3. Return to source ______________________ Signature

grid of line sources representing the urban area, and (2) a network of interstate and other major highways. A knowledge of the dispersion of particles emitted from a point source is basic to the development. The behavior in the system is determined by adding up or integrating the point source solutions over the line segment sources.

We are presently using a point source solution which has been developed by modifying the Gaussian dispersion equation to take into account gravitational settling. The solution is derived by solving a differential mass balance and it contains no arbitrary coefficients. The dispersion coefficients vary in the wind direction. The governing equations allow for a particle size distribution, each size range of which has its own settling characteristics. The total mass concentration and ground deposition rate are obtained by summing over the contributions of the various particle size ranges. We have assumed that the wind velocity is constant and that the diffusion coefficients are independent of height and lateral position. In addition, the solution does not adequately describe the conditions at the ground-air interface. Future solutions must correct these deficiencies.

We have integrated the point source solution over a line of arbitrary length: the angle between the wind direction and the line segment can have any value from zero to ninety degrees. We are presently applying these solutions to the region of interest. The rural area consists of a network of interconnected straight-line segments. The urban region is approximated by a rectangular grid of major thoroughfares over which the total urban lead output is distributed.

Two specific cases, viz., the point source and the line source with a wind normal to it, have been programmed on an IBM 360-75 computer.

In addition to the mathematical studies, it is envisaged that a limited number of field measurements using high-volume samplers will be made during this grant period.

E. A General Systems Approach to the Economics of Environmental Quality Management: An Analysis of the Removal of Lead Additives from Gasoline

In order to coordinate its plan of attack against automobile air pollution, the Environmental Protection Agency recently implemented a set of regulations on fuels and fuel additives. The new controls provide for the general availability of one grade of lead-free gasoline by July 1, 1974, and for a phased reduction in the lead content of the "regular" and "premium" grades during the period 1974 to 1977.

The EPA designed these regulations to improve environmental quality in the following ways: 1) the general availability of unleaded gasoline will complement the auto industry's strategy for compliance with the 1975-76 Federal emission standards; leaded gasolines are incompatible with the operation of the catalytic converter emission control systems that the major manufacturers are planning to install in the 1975 model vehicles. 2) the phased reduction in the lead content of gasoline is essential in order to reduce the present concentration of airborne lead which, in the Agency's judgment, constitutes a sufficient risk to the public's health.

Although these environmental protection controls will not generate measurable improvements in environmental quality for several years, the question must be raised concerning whether the prospective benefits of controlling the lead content of gasoline are likely to be greater than the estimated costs of compliance. To answer this question, the probable effectiveness of each regulation must be assessed and the benefits and costs of control must be quantified. In this manner it can be determined whether the economic and technological impositions of a specific regulation are too expensive to justify the benefits that will accrue to society as the result of a decision to enforce that control.

One of the dilemmas that the economist faces in making benefit-cost evaluations of environmental management decisions is that these decisions generally affect a large set of interdependent economic-technological-environmental relationships. As a framework of analysis, the scope of economic theory is too narrow to account for all of the important interactions that occur as an environmental management decision is enforced. Clearly, a methodology is needed wherein the elements of economics and other social sciences are integrated with the principles of engineering and natural science in a total systems approach.

The purpose of this section of the report is to develop amplified analytical methods for studying the economic impact of reducing the lead content of gasoline. The use of the systems approach facilitates expanding the framework of economic analysis so that it encompasses the interactions that may occur between different entities. In this context a system is defined as a framework for analyzing alternative reciprocal influences between entities whose behaviors are governed by diverse incentives and/or principles. Within the total system a decision to alter one set of dependent relationships, for example, waste emissions and environmental quality, may induce significant changes in all sets of interrelationships.

Because a total evaluation of the kind envisaged here is so complex, this discussion deals mainly with how the evaluation can be organized with only limited application of the organizational mode.

Figure 130 illustrates a systems framework for evaluating a set of alternatives or options for improving environmental quality. The important elements of this system are the constraints, (legal-political, environmental, technological, and economic) that determine the choice of an environmental management option and 2) affect the outcome of that decision. By defining the relevant components that shape the systems evaluation, the object of analysis, the environmental management decision, becomes embedded within the behavior of the system structure.

Defining the system produces an overall picture of what kinds of regulations are likely to be implemented and what is likely to happen after they go into effect. The systems approach provides an awareness of the flexible and of the resistant points, where acceptance or opposition to the desired change is likely to occur in the system. In short, defining the system facilitates the systems evaluation.

Consider Figure 133 as a checklist of studies that must be made in evaluating the impact of various management options to remove lead from gasoline. The numbered items correspond to the numbered entries in Figure 133. An analytical approach or method that is useful in making these studies is listed below each entry with an example of the kind of information that each study will produce.

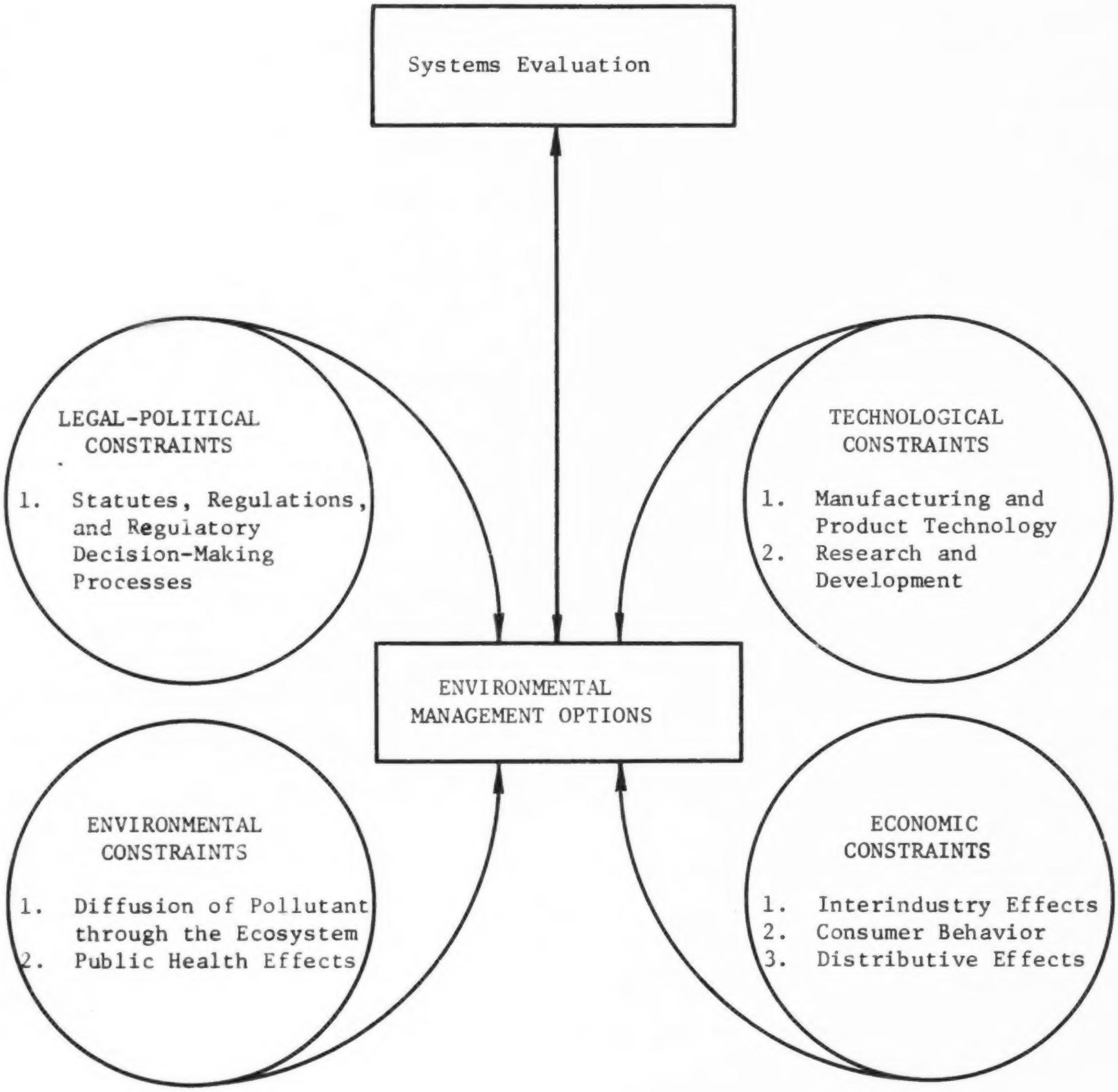

Systems Evaluation
LEGAL-POLITICAL CONSTRAINTS
1. Statutes, Regulations, and Regulatory Decision-Making Processes
TECHNOLOGICAL CONSTRAINTS
1. Manufacturing and Product Technology
2. Research and Development
ENVIRONMENTAL MANAGEMENT OPTIONS
ENVIRONMENTAL CONSTRAINTS
1. Diffusion of Pollutant through the Ecosystem
2. Public Health Effects
ECONOMIC CONSTRAINTS
1. Interindustry Effects
2. Consumer Behavior
3. Distributive Effects

Figure 132. Systems Evaluation Framework

A. LEGAL-POLITICAL CONSTRAINTS

1. Statutes,Regulations, and Regulatory Decision-Making Processes

   Structural analysis of government organization and of decision-making processes: -- predictions of government decisions.

2. Public Opinion and Voter Behavior

   Public opinion polls: -- estimates of the public's acceptance of environmental control.

B. ENVIRONMENTAL CONSTRAINTS

1. Diffusion of Pollutant through the Ecosystem

   Stochastic simulation analysis: -- predictions of the ecological effects of pollution and pollution control.

2. Public Health Effects

   Stochastic simulation analysis: -- predictions of the health effects of pollution.

C. TECHNOLOGICAL CONSTRAINTS

1. Manufacturing and Product Technology

   Engineering-economic analysis: -- estimates of the expenditures that will be required using current technology to change production processes and/or products in order to meet anti-pollution regulations.

2. Research and Development

   Technological forecasting: -- estimates of the expenditures that will be required to develop new technology for production processes and/or products in order to meet anti-pollution regulations.

D. ECONOMIC CONSTRAINTS

1. Interindustry Effects

   Input-output analysis: -- estimates of income and employment effects that result from shifts in allocation of resources.

2. Consumer Behavior

   Marketing analysis: -- estimates of changes in product demand.

3. Distribution Effects

   Incidence studies: -- estimates of impact of costs of pollution control by income class.

Figure 133. Systems Evaluation 'Checklist'

For example, eliminating the use of leaded gasoline will certainly create an interindustry impact (D.1) on a chain of related industries such as lead mining, smelting and refining, tetraethyl lead, oil refining, and construction. Input-output analysis is used to estimate the income and employment changes that will result from simultaneously decreasing the demand for leaded gasoline and increasing the demand for unleaded gasoline. These estimates then enter as one factor in the total systems evaluation.

In the following sections, the conclusions from some of the studies that are related to the removal of lead additives from gasoline are briefly summarized. The summary conforms to the checklist in Figure 133.

Before discussing each of the constraints in detail, it should be noted that the systems approach is very important to the field of environmental economics because of the urgent need to formulate a new analytical framework in that area. Several economists have rejected the post-war analytical treatment of pollution-related external effects because it does not provide an adequate framework for dealing with the problems of air and water pollution, urban sprawl, and other cancers of environmental decay. These writers have stressed that economic theory must be remolded if it is to furnish an effective framework for dissecting out the economic consequences of environmental control.

The basis for this rejection is that within the broader context of welfare economics, the traditional theory of externality has a conceptual bias that undermines its expediency to depict accurately the complexity and the pervasiveness of the pollution problem. Kneese and Ayres (1969), for example, have stated that there has been a tendency in the economics literature to consider all external interferences to production and consumption activities as exceptional situations. This tendency reflects a notion that the relationship between the externality and the achievement of allocative efficiency is equivalent to the relationship between the "fly" and the "ointment." As Mishan (1971) has stated:

> . . . , externalities today provide the standard exception to the equation of optimality with universal and perfect competition.

In a later article, Kneese (1971) presented an expanded discussion of some of the analytical roadblocks that stem from viewing the occurrence of external interference as an exceptional situation. First, external interference generally has been examined within a partial equilibrium framework as an isolated event or phenomenon. Second, externalities have been described in the simplified terms of a bilateral setting in which the source, the direction, and the consequences of the interaction have all been well understood.

Finally, the treatment of external effects as exceptional events has implied that simple ad hoc arrangements are effective in offsetting the interference and any of the associated distorting effects that the externality may have had on the allocation of resources.

Kneese (1971) has concluded that this kind of analysis is not at all analogous to the pervasive external effects that are characteristic of the pollution problem:

> . . . environmental pollution is not an isolated or random phenomenon, but in developed economies, at least, it must be regarded as inherent in man's production and consumption activities.

Kneese's prescription for what is needed includes reformulating economic theory so that it provides an appropriate setting for pollution-related externalities.

> . . . an analytical orientation which regards externalities as a systematic and pervasive phenomenon in modern economics

> is much more on all fours with the real situation than one which regards them as a somewhat freakish anomaly which can often, if not usually, be efficiently controlled by _ad hoc_ measures or private bargaining. (Kneese, 1971)

We want to stress that the systems approach is a methodology. Its strength is that it facilitates breaking-down the total configuration of interdependent relationships that are present in large-scale, complex social problems such as the pollution problem. A research project that employs the systems approach is obviously dependent upon an interdisciplinary team of experts who have a common interest in solving the problem of concern.

Legal-Political Constraints

A.1. Statutes, Regulations, and Regulatory Decision-Making Processes

The passage of the Clean Air Act of 1970 dramatically changed the federal government's role in controlling air pollution. This law gave the newly-created Environmental Protection Agency the authority to establish ambient air quality standards for the nation as a whole. Although the federal government had had the authority to establish automobile emission standards under the Clean Air Act of 1967, the 1970 law authorized the establishment of standards that would reduce present levels of emissions by 90 percent by 1975-76.

During the spring of 1971, the EPA held hearings for the purpose of translating the law into standards. The agency announced the results of these deliberations last June (Table 48).

It is important to assess these statutes and regulations from the standpoint of what it is they are attempting to control. An examination of automobile emission standards clearly indicates that the thrust of automobile air pollution control has been aimed at the tailpipe of the vehicle.

TABLE 48

Automobile Emissions Standards*

Federal	Prior to Control	1968	1970	1971	1973	1974	1975
HC	11.0	3.4	2.2	2.2	2.2	1.5	0.41
CO	80.0	34.0	23.0	23.0	23.0	23.0	3.40
NO_x	4.0				3.0	3.0	0.40
Evap. Contr.				YES			YES
Particulates	0.1-0.4						0.10

California	Prior to Control	1966	1970	1971	1972	1974	Proposed 1975
HC	11.0	3.4	2.2	2.2	1.5	1.5	0.5
CO	80.0	34.0	23.0	23.0	23.0	23.0	12.0
NO_x	4.0	--	--	4.0	3.0	1.3	1.0
Evap. Contr.	--	--	YES	YES	YES	YES	YES

*Emission Requirements in grams/mile

The EPA has largely neglected other major points of control such as the internal combustion engine and the automobile-dominated transport system that is deeply ingrained in our socio-economic system.

This in part reflects the results of several studies such as the 1966 U. S. Public Health Service Study which emphasized the characteristics of automobile air pollution instead of its causes. It was estimated that automobile emissions accounted for over 60 percent of the total tonnage of major air pollutants--63 percent of the hydrocarbons, 93 percent of the carbon monoxide, and 46 percent of the oxides of nitrogen--emitted each year in the United States (The Automobile and Air Pollution, 1967).

Because laws are not made within a vacuum, the emphasis on tailpipe control also reflects an organized effort on the part of the automotive industry to protect its economic and technological interests in the internal combustion engine. Bain (1970) has analyzed the auto industry's strategies for meeting the 1975-76 emission standards, and he states:

> By 1968 or 1969, with rapidly developing California and federal legislative action to regulate automotive air pollution, American auto makers were in a position that favored their developing a concerted general strategy for coping positively with regulatory constraints it faced or was about to face--a strategy that would put them in the driver's seat in selecting specific reactions to regulatory pressures, while still leaving room for about as much nonprice rivalry as they desired. The high concentration of the industry together with the existence of a common "opponent" made the adoption of such a positive strategy feasible.

In view of the predilections that have been built into the statutes, regulations, and regulatory processes, the controls that have been placed on fuels must be considered as complementing a strategy to control tailpipe emissions. Indeed, in announcing the regulations for fuel additives, the EPA stated that it had determined that the emissions products of gasolines that contain lead additives would impair significantly the performance of emission control systems such as the catalytic converters which the auto industry is developing to meet 1975-76 standards (Federal Register, 23 February 1972).

Because of the automobile industry's heavy reliance on the questionable technology of the catalytic converter, it is difficult to assess whether the controls--emission and fuel--that the EPA has stacked together will produce a net increase in social welfare. Bain (1970) concludes:

> . . . we may have a situation in which the choice that is most profitable or least unprofitable to automotive producers is different from the choice that would be most economical to the public. But if the decision is left up to the automotive producers the most profitable choice from their standpoint is likely to be made.

A.2. Public Opinion and Voter Behavior

Although there are not any existing studies on the impact of automobile emissions standards and gasoline controls on public opinion or voter behavior, the importance of the public's acceptance of these standards cannot be over-emphasized. Many laws that have been passed "in the public interest" were unenforceablesimply because the public did not support them. For auto emission standards, however, public opinion indicators will in all likelihood be very weak until after these standards go into effect in 1975-76.

Environmental Constraints

B.1. Diffusion of the Pollutant through the Ecosystem

A major interdisciplinary study of environmental diffusion of lead and other heavy metals is currently underway at the University of Illinois.

The progress of that study has been summarized in the first part of this report.

B.2. Public Health Effects

The EPA's decision to implement a phased reduction in the lead content of the "premium" and "regular" grades of gasoline was based on available medical evidence that airborne lead levels exceeding 2 micrograms per cubic meter, over a 3-month period or longer, create a sufficient risk of adverse health effects for the public. This conclusion was based on a summary study of the health hazards of lead (EPA, 1972); most of the technical material in this study had been summarized previously in the National Research Council-National Academy of Sciences (1971) study of airborne lead.

Four of the conclusions listed in the EPA's summary study serve to explain the agency's overall position on the health hazards of lead in the environment. These are:

1) Lead has not been shown to have any biologically useful function; all available data indicate that the metabolic effects of low concentrations of lead are of the inhibitory or adverse type. Accordingly, any increase in body burden of lead is accompanied by an increased risk of human health impairment.

2) Though the amounts of lead ingested in the diet generally are greater than the amounts inhaled, the body absorbs a greater percentage of inhaled lead 30% or more) than of ingested lead (about 10%).

3) Human blood lead levels begin to rise appreciably with exposure to airborne lead concentrations in excess of 2 micrograms per cubic meter.

4) Elevated lead intake for periods as short as, and possibly shorter than, three months is sufficient to produce an increase in blood lead levels.

Technological Constraints

C.1. Manufacturing and Product Technology

Several studies have been made to estimate the additional costs involved in eliminating the use of lead in gasoline production. Adding tetraethyl lead to refined gasoline has been the cheapest way for the producer to increase the octane rating, the 6-8 octane numbers that are necessary for high-octane high-compression engine fuels. Without lead additives, refiners must substitute more costly components such as aromatics to increase octane ratings to usable levels.

The Bonner and Moore (1971) study is the most comprehensive economic analysis of the impact of lead removal on the refining industry. The study, which was conducted for the EPA, is a feasibility analysis of fifteen different lead removal strategies. Each strategy was examined to determine what changes it would produce on capital costs, pool octane numbers, aromatics concentrations, and prime blending component requirements. The study also accounted changes in annual gasoline consumption through 1980.

The main conclusions of the study are:

1) A rapid phased reduction will require a large buildup of refinery construction activity that will reach a sharp peak and then decline quickly.

2) The added per gallon costs of lead removal are not large. The per gallon costs of the phased reduction schedule that the EPA has adopted (see Table 49) are approximately .25 cents per gallon.

3) Controlling the lead levels of conventional grades of gasoline determines how fast lead can be removed if the attrition of older cars is the only removal mechanism. A rapid lead removal schedule requires a larger capital investment than a slow removal schedule; the schedule that the EPA has adopted will require an additional capital investment of nearly $2.5 billion by 1980.

TABLE 49

Environmental Protection Agency Phased Reduction Schedule*

Grade Octane	1974	1975	1976	1977	1978	1978	1980
93	0.00	0.00	0.00	0.00	0.00	0.00	0.00
94	2.00	1.70	1.50	1.25	1.25	1.25	1.25
100	2.00	1.70	1.50	1.25	1.25	1.25	1.25

*TEL contents of gasolines (g/gal.)

C.2. Research and Development

One of the largest gaps in the present state of economic and technological knowledge on automobile air pollution control consists of the lack of economic and technological forecasts for products that may replace 91-octane unleaded gasoline, the internal combustion engine, the catalytic reactor system or any combination of these. The only entry in this area is a Universal Oil Products (1971) study of the economic and technological impact of producing 100-octane unleaded gasoline on refinery operations. Clearly, much more research is needed in this area.

Economic Constraints

D.1. Interindustry Effects

Hartman (1971) of the Colorado State University project has used Leontief input-output analysis to estimate the income and employment effects of reducing the refining industry's demands for tetraethyl lead to zero. Hartman has concluded that the income effect will be approximately $317 million and employment effect will be about 12,000 workers put out of work.

As indicated in the next section (D.2.), these effects will occur very slowly over the next eight to ten years. In addition, the growth of the refinery construction industry and the growth of industries involved in producing the catalytic converter system will, in all likelihood, more than compensate for the losses to the tetraethyl lead complex. In other words, the net interindustry income and employment effects that stem from automobile air pollution control will be positive.

D.2. Consumer Behavior

One of the most critical and one of the most overlooked areas of research on the economic impact of environmental control concerns the consequences of the consumers' willingness to purchase new "environmentally-sound" products. For example, thus far consumer acceptance of unleaded or low-lead gasoline has been very poor; low-lead sales account for less than 5 percent of the volume of gasoline sold (Business Week, 1971).

Because the total automobile population is increasing, the total amount of leaded gasoline being purchased is also increasing. If total lead content of gasoline is accepted as a proxy variable for total lead emissions, then lead emissions are also increasing (see Figure 134).

The tonnage figures presented in Figure 134 are annual estimates of the total lead content of gasoline. These data are based on estimates of total vehicle miles driven by cars and trucks each year. The total vehicle mile data were calculated from a set of equations provided by EPA (Bonner and Moore, 1971).

Total vehicle miles were then broken down into three categories in order to estimate annual consumption of gasoline: 1) car and truck miles for 1970 and earlier model vehicles; 2) car and truck miles for 1971 through 1974 model vehicles; and 3) car and truck miles for post-1975 model vehicles.

The Bonner and Moore assumptions on average miles per gallon, on the percentage of total miles driven by each vehicle age group, and on consumer purchasing patterns for the different grades of gasoline were used to estimate the number of gallons of regular, premium, and unleaded grades of gasoline that would be purchased each year. For the 1971-through-1974 model year

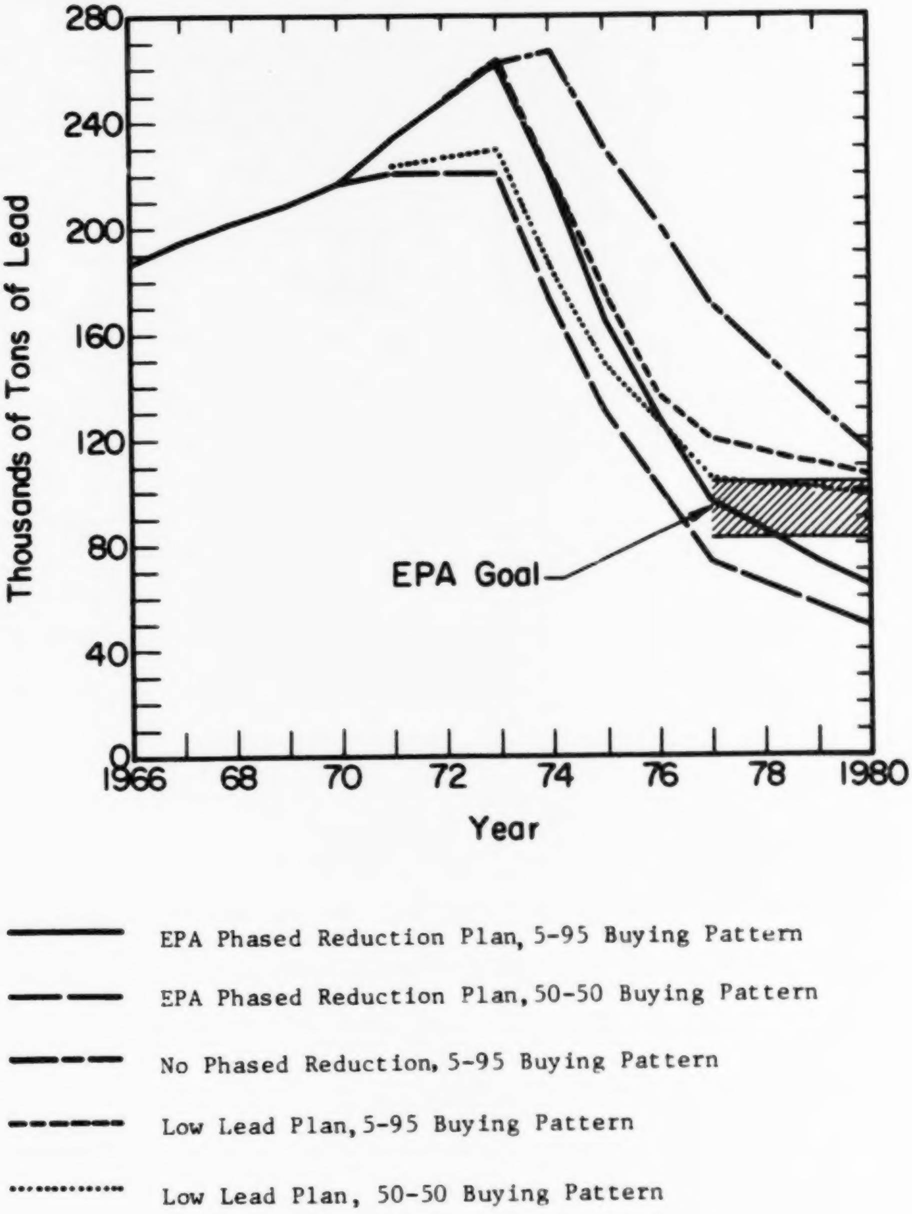


Figure 134. Gasoline-Lead Requirements, 1966-1980

vehicles the purchasing pattern for regular and unleaded gasoline was varied to simulate the consequences of different consumer reactions to the use of unleaded gasoline.

To obtain estimates of total gasoline-lead requirements, the total gallon figures for the regular, premium, and unleaded grades were multiplied by the tetraethyl lead content figures shown in Table 50. A total gasoline-lead requirement was also estimated for a strategy that included a low-lead (.500 g/gal) grade of gasoline. Selected trends in the reduction of gasoline-lead consumption are illustrated in Figure 134. These trends indicate:

1) Gasoline-lead consumption will continue to rise through 1973. This rise is mainly due to consumer resistance to the use of low-lead or unleaded gasolines that are now on the market.

2) The EPA's phased reduction plan will decrease gasoline-lead consumption significantly. However, this decrease will not be completely insensitive to consumer purchasing patterns.

3) If gasoline-lead consumption can be accepted as a proxy for airborne lead concentrations, the EPA's goal of reducing present airborne lead levels by 60-65 percent will not be achieved until 1977-78.

4) Finally, alternative strategies, such as a less expensive low-lead plan may reduce gasoline-lead consumption almost as rapidly as the EPA's no-lead plan.

D.3. Distributive Effects

The question of who will pay for automobile air pollution control has not been approached at all. Incidence studies on the impact of the costs of pollution control on different income classes are badly needed in order to assess distributive effects of environmental management decisions.

TABLE 50

Tetraethyl Lead Content

(g/gal)

	EPA Phased Reduction Plan		No Phased Reduction	
Year	Regular	Premium	Regular	Premium
1970	1.935	2.738	1.935	2.738
1971	2.150	2.738	2.150	2.738
1972	2.262	2.794	2.262	2.794
1973	2.373	2.815	2.373	2.815
1974	2.000	2.000	2.373	2.815
1975	1.700	1.700	2.242	2.937
1976	1.500	1.500	2.168	2.690
1977	1.250	1.250	2.168	2.690
1978	1.250	1.250	2.168	2.690
1979	1.250	1.250	2.168	2.690
1980	1.250	1.250	2.168	2.690

To conclude, the checklist summary indicates that there are several entries that contain little or no information on the impact of removing lead from gasoline. Although some of these entries will be filled in the near future, a large amount of research will be needed, especially in the area of quantifying the costs and benefits of automobile pollution control, before a total systems evaluation can be made.

F. Personnel

Transport of Lead in the Environment

Dr. J. Melin
Dr. G. Rolfe
Dr. A. Haney
Amar Chaker
Clayton Weaver

Dynamic Model of Lead Flow in the Body

Jamal Abdel-Nour
Dr. R. Forbes

Plant Modeling

Dr. G. Rolfe
Jorge Garcia
Dr. A. Haney

Atmospheric Transport

Dr. J. L. Hudson
Dr. J. Stukel
Timothy Crawford

A General Systems Approach to the Economics of Environmental Quality Management

Dr. G. Provenzano
Dr. R. Woodhead

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Mishan, E. J. "The Postwar Literature on Externalities: An Interpretative Essay", JOURNAL OF ECONOMIC LITERATURE 9, No. 1, pp. 1-28 (March 1971).

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Universal Oil Products Company, "Manufacturing Tomorrow's Gasolines" (L. O. Stine, R. M. DeVeirman, and R. P. Schuller) presented at the annual meeting of the National Petroleum Refiners Association (21-23 March 1971).

IX. APPENDIX

A. PERSONNEL

1. Administration

Coordinating Committee

B. B. Ewing, Professor of Sanitary Engineering; Director, Environmental Studies Program

H. A. Laitinen, Professor of Chemistry; Director, Metals Project

W. R. Boggess, Professor of Forestry; Head of Department

J. B. Hanson, Professor of Plant Physiology; Head of Department of Botany

R. L. Metcalf, Professor of Zoology and Entomology; Head of Department of Zoology

J. T. Pfeffer, Professor of Sanitary Engineering; Member, Advisory Panel on Pollution Abatement Policies

G. W. Salisbury, Professor of Dairy Science; Director, Agricultural Experiment Station

B. W. Carnow, Head of Department, Preventive Medicine, Lincoln School of Medicine, Chicago

Steering Committee

H. A. Laitinen, Chairman; Air-Soil-Water-Plant Continuum Group
B. B. Ewing, Modeling Group
A. M. Hartley, Analytical Chemistry Laboratory
G. L. Rolfe, Total Ecosystem Group
F. A. Bazzaz, Plant Processes Group
R. M. Forbes, Animal Processes Group

Administration

H. A. Laitinen, Task Force Director
W. von Fischer, Assistant Director
D. Abernathy, Secretary
B. Blanding, Clerk-Typist

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2. Analytical Chemistry Laboratory

Faculty Participant
- H. A. Laitinen, Professor of Chemistry

Laboratory Director
- A. M. Hartley

Assistant Director
- R. S. Vogel

Research Assistants
- L. M. Chessmore
- R. C. Fry
- J. C. Johnston
- N. L. Puckett
- N. Watkins

3. Total Ecosystem

Group Leader
- G. L. Rolfe, Assistant Professor of Forestry

Faculty Participants
- J. D. Alexander, Associate Professor of Pedology
- W. R. Boggess, Professor of Forestry
- O. C. Braids, Assistant Professor of Soil Organic Chemistry
- L. L. Getz, Professor of Zoology
- A. W. Haney, Assistant Professor of Botany
- R. W. Larimore, Professor of Environmental Biology
- H. V. Leland, Assistant Professor of Environmental Biology
- R. L. Metcalf, Professor of Zoology and Entomology
- P. W. Price, Assistant Professor of Entomology
- G. C. Sanderson, Professor of Zoology
- H. G. Wenzel, Associate Professor of Civil Engineering
- M. F. Willson, Associate Professor of Zoology
- R. H. Wortman, Assistant Professor of Transportation

Research Assistants
- J. A. Baker
- J. M. Edgington
- B. Poole
- L. Verner
- V. C. Weaver

Technical Assistants
- A. Brigham
- J. McNurney
- J. L. Nelson
- R. Richart
- M. J. Wetzel

Student Help
- D. D. Anderson
- K. Moller

4. Air-Soil-Water-Plant Continuum

Group Leader
- H. A. Laitinen

Faculty Participants
- O. C. Braids, Assistant Professor of Soil Organic Chemistry
- D. E. Koeppe, Assistant Professor of Plant Physiology
- H. V. Leland, Assistant Professor of Environmental Biology
- R. J. Miller, Professor of Soil Physical Chemistry

Research Associate
- R. R. Gadde

Research Assistants
- J. E. Bittell
- K. W. Boyer (M.S.P.H. Fellow)
- F. J. Drone
- R. G. Weber

Student Help
- B. Wuebbles

5. Plant Processes

Group Leader
- F. A. Bazzaz, Assistant Professor of Botany and Forestry

Faculty Participants
- Dr. Govindjee, Professor of Botany and Biophysics
- J. B. Hanson, Professor of Plant Physiology
- D. E. Koeppe, Assistant Professor of Plant Physiology
- R. J. Miller, Professor of Soil Physical Chemistry

Research Associates
- M.D.L. Bakri
- R. A. Root

Research Assistants
- R. B. Hummeland
- P. N. Windle

6. Animal Processes

Group Leader
- R. M. Forbes, Professor of Nutritional Biochemistry

Faculty Participants
- R. C. Hiltibran, Associate Professor of Agronomy
- G. L. Jendrasiak, Assistant Professor of Physiology and Biophysics
- D.F.S. Natusch, Assistant Professor of Chemistry
- J. Simon, Professor of Veterinary Pathology
- A. R. Twardock, Professor of Veterinary Physiology

Research Associate
- R.L.C. Kao

7. Modeling Team

Team Coordinator
- B. B. Ewing

Team Leader
- G. L. Rolfe

Faculty Participants
- F. A. Bazzaz, Representing Plant Processes
- O. C. Braids, Representing Soil Chemistry
- R. M. Forbes, Representing Animal Processes
- A. W. Haney, Representing Plant Ecology
- J. L. Hudson, Associate Professor of Chemical Engineering
- J. W. Melin, Associate Professor of Civil Engineering
- J. E. Pearson, Professor of Sanitary and Nuclear Engineering and Assistant Director of Environmental Studies Program
- J. J. Stukel, Associate Professor of Mechanical and Civil Engineering
- R. W. Woodhead, Professor of Civil Engineering

Research Associate
- G. Provenzano

Research Assistants
- J. G. Abdel-Nour
- A. A. Chaker
- J. Garcia

Graduate Students
- T. Crawford
- J. Wedding

B. SPECIAL PROJECT SEMINARS

November 17, 1971	Characterization of Automotive Particulates Dr. K. Habibi, E. I. duPont de Nemours & Co., Inc. Wilmington, Delaware Atmospheric Chemistry of Vehicle Exhaust Particulates Dr. J. M. Pierrard, E. I. duPont de Nemours & Co., Inc. Wilmington, Delaware
January 19, 197	Precipitation Scavenging Mechanism R. G. Semonin, Precipitation Physics Group, Atmospheric Science Center, Illinois State Water Survey
February 16, 1972	Total Ecosystem Mass Transport Model J. W. Melin, Associate Professor of Civil Engineering, University of Illinois Gary L. Rolfe, Assistant Professor of Forestry, University of Illinois
March 1, 1972	Colorado State University Study of Environmental Contamination Caused by Lead H. W. Edwards, Associate Professor of Mechanical Engineering, Principal Investigator and Program Coordinator, CSU Lead Project
March 15, 1972	Inhibition of Photosystem II of Photosynthesis by Lead Donald Miles, Assistant Professor, University of Missouri, Columbia
April 19, 1972	Cellular Pathology of Lead in Mammalian Systems Dr. Robert A. Goyer, School of Medicine, Department of Pathology, University of North Carolina, Chapel Hill

C. TRACE METALS ANALYSIS SYMPOSIUM, JANUARY 7 and 8, 1971

January 7, Morning

Introductory Remarks
H. A. Laitinen

Activation Analysis for Trace Metals
W. S. Lyon, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Spectrochemical Methods
R. K. Skogerboe, Colorado State University

Mass Spectrometer for Organo-Lead Systems
R. Dougherty, Florida State University

Chelates and GC Techniques for Metals
R. A. Keller, State University of New York, Fredonia

Electroanalytical Methods
W. D. Shults, Oak Ridge National Laboratory

X-ray Fluorescence for Determination of Lead in Paint
T. J. Kneip, New York University

January 7, Afternoon

Discussion Session I - Mercury Methods
Leader: V. A. Stenger, The Dow Chemical Co., Midland, Michigan
Panel: Charles (Drew) Evans, Senior Research Chemist, Materials Research Laboratory, U. of I.
D. G. Langley, T. W. Beak Consultants, Toronto, Canada
J. M. Wood, Associate Professor of Biochemistry, U. of I.
R. G. Smith, School of Public Health, University of Michigan

Discussion Session II - Intercomparison of Analytical Techniques
Leader: J. C. White, Oak Ridge National Laboratory
Panel: E. P. Pryzbylowicz, Eastman Kodak Co., Rochester, New York
R. K. Skogerboe
Bryan Madison, Procter and Gamble Co., Cincinnati, Ohio

Group Dinner
Speaker: Discrimination Factors Among Metals During Their Transfer Between Related Phases of an Ecological System
C. C. Patterson, California Institute of Technology

January 8, Morning

Standard Materials for Trace Metals Determination
W. Wayne Meinke, National Bureau of Standards

Lead in Milk, an Interlaboratory Comparison
Manuel Brandt, The Ethyl Corporation

Atmospheric Lead - Intermethods and Interlaboratory Comparison
J. O. Pierce, University of Missouri

Simultaneous Determination of Heavy Metals in Environmental Samples
Dr. Anna M. Yoakum, Stewart Laboratories, Knoxville, Tennessee

Direct Determination of Metals in Airborne Particulates
E. P. Pryzbylowicz

The Role of Complexation in Trace Metal Transport in the Environment
Reginald Griffin, Environmental Science Association, Newtonville, Massachusetts

January 8, Afternoon

Discussion Session III - Sampling, Standard Methods, and Standard Materials; Interlaboratory Comparisons
Leader: W. Wayne Meinke
Panel: J. O. Pierce
R. K. Skogerboe
Gary TerHaar, The Ethyl Corporation
Michael D. Gebott, Poisonlab, Inc., Denver

Discussion Session IV - Looking Ahead
Leader: R. A. Keller
Panel: C. C. Patterson
J. O. Pierce
T. J. Kneip

D. SECOND TRACE METALS SYMPOSIUM
NOVEMBER 18 and 19, 1971

November 18, Morning	Introductory Remarks H. A. Laitinen
	Application of ESCA to Environmental Problems L. D. Hulett, Oak Ridge National Laboratory, Oak Ridge, Tennessee
	Trace Analysis for Metal Pollutants by GC R. E. Sievers, Wright Patterson Air Force Base
	Discussion Session I - Standard Reference Materials Panel Leader: F. D. LaFleur, National Bureau of Standards Panel Members: L. C. Hall, National Bureau of Standards (on leave from Vanderbilt University); J. Benton Jones, University of Georgia
November 18, Afternoon	Discussion Session II - Standardization and Validation of Analytical Methods Panel Leader: W. D. Shults, Oak Ridge National Laboratory Panel Members: N. F. Shimp, Illinois State Geological Survey; John K. Taylor, National Bureau of Standards; M. W. Skougstadt, United States Geological Service, Denver; Earl McFerren, Bureau of Water Hygiene Environmental Protection Agency, Cincinnati, Ohio; George Kupchik, New York
	Discussion Session III - Sampling, Sample Treatment, and Storage Panel Leader: Walter C. McCrone, Walter C. McCrone Associates, Inc., Chicago Panel Members: Gary Ter Haar, The Ethyl Corporation; Mark S. Shuman, University of North Carolina, School of Public Health; Charles A. Evans, Senior Research Chemist, Materials Research Laboratory, University of Illinois
	Dinner and Evening Address - Trace Analyses on Ultramicro Samples Walter C. McCrone

November 19, Morning

Use of Polythiol Resins to Accelerate Removal of Methyl Mercury Compounds from Animal Tissue
Hamish Small, The Dow Chemical Co., Midland, Michigan

Characterization of Airborne Lead
E. S. Jacobs, The E.I. du Pont de Nemours & Co., Inc.

Electrochemical Characterization of Trace Metals Interactions in Aquatic Environments
T. A. O'Shea, H. E. Allen, K. H. Mancy, University of Michigan

Teratology of Heavy Metals
T. A. Becker, University of Iowa

Cadmium and Hypertension
H. Mitchell Perry, Washington University, School of Medicine, St. Louis

November 19, Afternoon

Discussion Panel IV - Studies of Trace Metals in Water - Soil - Plant Continuum
Panel Leader: R. J. Miller, Professor of Soil Physical Chemistry, Department of Agronomy, University of Illinois
Panel Members: F. J. Stevenson, Professor of Soil Chemistry, Department of Agronomy, University of Illinois; Olin C. Braids, Assistant Professor of Soil Organic Chemistry, Department of Agronomy, University of Illinois; G. L. Jendrasiak, Assistant Professor of Physiology and Biophysics, University of Illinois; R. A. Durst, National Bureau of Standards

Discussion Panel V - Human and Animal Effects of Trace Metal Pollutants
Panel Leader: R. M. Forbes, Professor of Nutritional Biochemistry, Department of Animal Science, University of Illinois
Panel Members: H. E. Allen, B. A. Becker, L. C. Hall, H. Mitchell Perry

E. SEMINAR SERIES--CHEMISTRY AND BIOLOGY OF TRACE METALS IN THE ENVIRONMENT

SPRING SEMESTER, 1971

February 11	Chemistry of Trace Metals in the Environment H. A. Laitinen, Professor of Chemistry, University of Illinois
February 18	Methylation of Heavy Metals J. M. Wood, Associate Professor of Biochemistry, University of Illinois
February 25	Trace Metals - Cadmium, Lead and Copper - in the Blood and Serum of Cadmium - Dosed Rats R. Griffin, Environmental Sciences Associates, Inc., Cambridge, Massachusetts
March 4	Trace Metals in Soils O. C. Braids, Assistant Professor of Soil Organic Chemistry, Department of Agronomy, University of Illinois
March 11	Heavy Metals as Environmental Pollutants R. L. Metcalf, Professor of Zoology and Entomology; Head of Department of Zoology, University of Illinois
March 18	The Ion Microprobe M. Bayard, Walter C. McCrone Associates, Inc., Chicago
March 25	Trace Elements and Plant Growth R. J. Miller, Professor of Soil Physical Chemistry, and D. E. Koeppe, Assistant Professor of Plant Physiology, Department of Agronomy, University of Illinois
April 1	Trace Elements in Southern Lake Michigan H. V. Leland, Assistant Professor of Environmental Biology and of Zoology, University of Illinois, and N. F. Shimp, Head of Section of Analytical Chemistry, Illinois State Geological Survey
April 15	The Clinical Chemist's Responsibility in Environmental Health Dr. George Bowers, Jr., Director of Clinical Chemistry Laboratory, Hartford Hospital, Hartford, Connecticut

April 22	Some Coordination Compounds in Biochemistry J. C. Bailar, Jr., Professor of Chemistry, University of Illinois
April 29	Automation and Computerization of Analytical Measurements J. R. DeVoe, Institute of Materials Research, Washington, D.C.
May 6	Trace Metals in Coal H. J. Gluskota, Geologist, Illinois State Geological Survey
May 13	Trace Metals and Animal Growth and Longevity R. M. Forbes, Professor of Nutritional Biochemistry, Department of Animal Science, University of Illinois
May 20	Removal of Trace Metals from Public Water Supplies B. B. Ewing, Professor of Sanitary Engineering, Department of Civil Engineering; Professor of Nuclear Engineering; Director of Water Resources Center and of Environmental Studies Program, University of Illinois
May 27	Water Quality and Effluent Standards for Trace Metals S. R. Aldrich, Member Illinois Pollution Control Board; on leave as Professor of Soil Fertility Extension, Department of Agronomy, University of Illinois

F. LEAD PROJECT - PROGRAM REVIEW SYMPOSIUM

May 16, 1972

- Opening Remarks
  - H. A. Laitinen
- Environmental Research Laboratory, Analytical Facility
  - Capabilities Expansion
    - A. M. Hartley
  - Methods Development
    - A. M. Hartley
  - Cathodic Stripping Coulometry
    - N. Watkins
  - Routine Processing Activities
    - R. S. Vogel
- Total Ecosystem Studies
  - Input Measures, Output Measures, and Soil-Plant Studies
    - G. L. Rolfe and A. W. Haney
  - Animal Studies
    - L. L. Getz
  - Aquatic Studies
    - R. W. Larimore
- Air-Water-Soil-Plant Continuum Studies
  - Lead in Soils
    - O. C. Braids
  - Lead Uptake and Effects on Plant Physiological Processes
    - D. E. Koeppe
  - Pulse Polarographic Studies of the Interaction of Lead Ions with Clays, Plant Roots, and Root Exudates
    - R. Gadde
  - Characterization of Lead Halide Aerosol Particles by Electron Microscopy
    - K. W. Boyer
- Plant Processes Studies
  - Effects of Lead on Whole Plant Photosynthesis, Respiration, and Transpiration of Dominant Species in the Watershed Ecosystem
    - R. A. Bazzaz and G. L. Rolfe

Effect of Lead Salts on Photosynthetic Reactions in Isolated Chloroplasts
Dr. Govindjee and Maarib Bakri

Animal Processes Studies

Effects of Lead on Some Aspects of the Heme Synthetic Pathway
R. M. Forbes

Rates of Lead Accumulation in Adult Rats Fed Diets Containing Different Lead Concentrations
R. M. Forbes

The Interaction of Lead with Membranes and Membrane Lipids
G. L. Jendrasiak

Effect of Lead Species on Model Membranes
D.F.S. Natusch

Modeling Team Reports

Transport of Lead in the Environment
G. L. Rolfe, J. W. Melin, and A. Chaker

Dynamic Model of Lead Flow in the Body
J. Abdel-Nour

A General Systems Approach to the Economics of Environmental Quality Management: An Analysis of the Removal of Lead Additives from Gasoline
G. Provenzano

G. PROJECT-RELATED LECTURES AND PUBLICATIONS

Lectures

J. D. Alexander participated in the 1972 Illinois Fertilizer Conference sponsored by the U. of I. and the Illinois Fertilizer and Chemical Association in Springfield on January 5 and 6 and presented a paper on "Lead in Illinois Agriculture."

H. A. Laitinen spoke on "An Interdisciplinary Study of Environmental Pollution by Lead and Other Metals" at a seminar meeting of the Department of Chemical Engineering, U. of I., on February 22 and at a meeting of the Illinois State Geological Survey on February 25.

K. W. Boyer presented an Analytical Chemistry seminar on "Electron Probe Microanalysis With Some Recent Applications" on March 10.

R. M. Forbes spoke on "Biological Effects of Lead in Animals" at a seminar meeting, Department of Nutrition, School of Hygiene, University of Toronto, on March 30.

H. A. Laitinen spoke on "Analytical Chemistry in Environmental Science" at a meeting of the Analytical Topics Group, Cleveland, Ohio, section of the American Chemical Society on April 5.

H. A. Laitinen spoke on "The Role of Chemistry in Environmental Science" at a Phi Lambda Upsilon Meeting in Miniature at Purdue University on April 28.

H. A. Laitinen spoke on the "Determination of Lead by Cathodic Stripping Coulometry," in a session on Electrochemical Contributions to Environmental Protection at the meeting of the Electrochemical Society in Houston, Texas on May 9.

Papers Published

"Simultaneous Determination of Bromine and Chlorine with Methyl Orange" by H. A. Laitinen and K. W. Boyer, ANALYTICAL CHEMISTRY, Vol. 44, No. 6, May 1972, pp. 920-926.

"Chlorophyll Fluorescence and Photosynthesis Fluorescence Transients", by Govindjee and G. Papageorgiou, PHOTOPHYSIOLOGY, Vol. 6, 1971, p 1.

"Thermoluminescence and Temperature Effects on Delayed Light Emission (Corrected for Changes in Quantum Yield of Fluorescence) in DCMU-Treated Algae" by P. Jursinic and Govindjee, PHOTOCHEM. PHOTOBIOL., Vol. 15, 1971, p. 331.

"Lead Effects on Corn Mitochondrial Respiration" by D. E. Koeppe and R. J. Miller, SCIENCE, Vol. 167, 1970, pp. 1376-1378.

"Some Effects of Decenylsuccinic Acid on Isolated Corn Mitochondria" by D. E. Koeppe and R. J. Miller, PLANT PHYSIOL., Vol 48, 1971, pp. 659-662.

"Proton Magnetic Resonance Studies of Metal Complex Formation in some Sulphur-containing Alpha-Amino Acids" by D. F. S. Natusch and L. J. Porter, JOURNAL OF CHEMICAL SOCIETY A, 1971, p. 2527.

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"Lead Pollution in a Watershed Ecosystem" by B. B. Ewing, G. L. Rolfe and J. W. Melin to be presented at a Symposium on the Impact of Heavy Metals on Ecosystems at a meeting of the Ecological Society of America to be held jointly with the annual meeting of the American Institute of Biological Sciences in Minneapolis, August 27 - September 4.

"Lead Distribution and Movement in a Woodland Ecosystem" by G. L. Rolfe and W. R. Boggess.